INFLUENCE OF CIRCADIAN MISALIGNMENT ON SLEEP, ENERGY

EXPENDITURE, THERMOREGULATORY PHYSIOLOGY, AND COGNITION

by

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ABSTRACT

Andrew W. McHill (Department of Integrative Physiology)

Influence of Circadian Misalignment on Sleep, Energy Expenditure, Thermoregulatory Physiology and Cognition.

Thesis directed by Associate Professor Kenneth P. Wright, Jr.

Demands of modern society force many work operations into the late night when the intrinsic circadian timing system is promoting sleep. Overnight shiftwork is associated with increased risk for adverse metabolic health, accidents and injury, and sleep disruption. Uncovering potential physiological mechanisms by which working and eating at adverse circadian times contribute to metabolic dysregulation are vital to the development of treatment strategies. Furthermore, understanding the time course of cognitive performance decrements during shiftwork and mechanisms by which caffeine improves alertness and disrupts sleep are important for developing evidence-based fatigue management approaches. Therefore, the aims of this dissertation were to: 1) determine fundamental changes in physiology that would promote a state of unwanted weight gain in response to food consumed during simulated overnight shiftwork; 2) determine how cognitive performance changes during extended wakefulness on the transition to the first night shift and during subsequent night shifts; and 3) determine if early morning caffeine administration influences the distal-proximal skin temperature gradient and improves alertness while subsequently disrupting daytime recovery sleep.

The results indicate: 1) working during the night increased total daily energy expenditure (EE) on the transition to the first nightshift day and decreased total daily EE on the second and third nightshifts, decreased EE during daytime sleep episodes, decreased EE in response to a late dinner meal, increased total daily fat utilization on the first and second nightshifts and reduced carbohydrate and protein utilization on the second nightshift, and decreased subjective hunger

despite concurrent decreases in satiety hormones; 2) working during the night increased sleepiness and decreased cognitive performance which predominately did not change across subsequent nightshifts; and 3) caffeine administered 5h prior to daytime recovery sleep significantly decreased the distal-proximal skin temperature gradient, increased alertness, and disrupted daytime recovery sleep.

These findings suggest that reduced energy expenditure during nightshift work may represent a contributing mechanism by which working and eating during the night increases the risk of weight gain and obesity. Furthermore, results from this dissertation expand our knowledge on cognitive performance decrements across subsequent night shifts and physiological mechanisms by which caffeine improves alertness and disturbs sleep.

DEDICATION AND ACKNOWLEDGMENTS

I would like to dedicate this dissertation to all of my family and friends who have supported me throughout my entire education leading up to this PhD. Specifically, I dedicate this dissertation to my grandfather who inspired me to give thanks and appreciate all that is around me, to my father who taught me hard work and dedication, my mother who always pushed me and taught me the value of education, to my siblings who always forced me to be competitive and give my all, and especially to my wife Madeleine, who has supported and loved me during both the difficult and fun times.

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CHAPTER 1

EFFECTS OF CIRCADIAN MISALIGNMENT ON SLEEP,

ENERGY METABOLISM, AND THERMOREGULATORY PHYSIOLOGY

Andrew W. McHill

Introduction

The coordination and timing of specific behaviors and cellular processes in the brain and body are necessary for optimal functioning and are therefore fundamental to human physiology. At a cellular level, internal circadian clocks of the human brain and body have evolved to synchronize to the natural light-dark cycle of the 24h day, promoting wakefulness behavior and activities during the light period of the day and sleep during the dark period of the night. With relatively recent technological advancements such as electrical lighting and jet travel, wakefulness activities have been pushed later into the night and humans can now rapidly cross into places with differently timed light-dark cycles. However important these advancements are for society, they are not without a cost since the altered timing of these behaviors necessarily affect the endogenous physiological timing mechanisms responsible for promoting optimal functioning and health; a state of "circadian misalignment".

Over the last two decades, the links between weight gain, obesity, and disease have come to the forefront of health and scientific interest. According to the Center for Disease Control (CDC), over one third (35.7%) of Americans are obese (Ogden et al, 2012), leading to increased risk for cardiovascular disease, stroke, insulin resistance, and cancer. Explanations such as over-eating, poor nutritional diet choices, and insufficient activity cannot fully account for the continued rise in weight gain and poor health outcomes. Therefore, other potential risk factors must be examined to help account for this discrepancy. One such risk factor may be circadian misalignment (Markwald & Wright, 2012), as studies of people working rotating and permanent overnight shifts show increased risk for higher BMI (Di Lorenzo et al, 2003), metabolic syndrome (Canuto et al, 2012), circadian disorders (Drake et al, 2004), sleep loss (Torsvall et al, 1989; Åkerstedt, 2003; Åkerstedt & Wright, 2009) and impaired cognition (Tilley et al, 1982).

This comprehensive review will explore the existing literature detailing the relationship of the circadian system with sleep, energy metabolism, and thermoregulation. A specific emphasis will be placed on the outcomes of circadian misalignment in these systems. To begin, the regulation of physiology by both the circadian system and sleep and wakefulness will be reviewed. This will be followed by an examination of energy metabolism, highlighting its fundamental relationship with both the circadian system and sleep and wakefulness. This review will conclude with an investigation of thermoregulation and its interaction with sleep, the circadian system, and energy metabolism.

The Circadian System

In the early 1700's, astronomer and geophysicist Jean-Jacques d'Ortous de Mairan documented what is thought to be the first observations of endogenous 24h rhythms. In a simple experiment, de Mairan placed a *Mimosa* plant in constant darkness and observed an opening of the leaves during the day and a subsequent closing of the leaves during night (Moore-Ede et al, 1982; Meijer & Rietveld, 1989). With no input from the environment, it was determined that the plant had a self-sustained endogenous signal promoting the leaves to open during the day for photosynthesis and close at night to preserve energy (Moore-Ede et al, 1982). A 24h endogenous oscillation that is free running in the absence of environmental time cues, entrainable to environmental stimuli, and can compensate when placed in varying temperatures is termed a circadian rhythm (derived from the Latin terms *circa*: approximately, *dies*: day). Like the *Mimosa* plant, most organisms from single-celled cyanobacteria (Kondo et al, 1993; Nakajima et

al, 2005; Rust et al, 2011) to humans have evolved circadian rhythms to promote physiology and behaviors that are optimal during either the solar day or night.

In mammals, the circadian timing system is controlled by a central pacemaker, or master clock, located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus (Stephan & Zucker, 1972; Inouye & Kawamura, 1979). The SCN is composed of approximately 20,000 neurons acting as self-contained oscillators of a near 24h rhythm (Welsh et al, 1995; Ralph et al, 1990; Meijer et al, 1997). In rodent studies both *in vitro* and *in vivo*, the SCN increases neuronal firing rates (Meijer et al, 1997), glucose uptake (Schwartz et al, 1983), and gene expression (Gachon et al, 2004) during the subjective day to promote active states. Furthermore, SCN neurons use both neuronal and humoral outputs to project to peripheral clocks located throughout the body (e.g. kidneys, gut, pancreas, etc.). Thus these peripheral oscillating clocks are synced with the master clock so that biological functions are organized for anticipation of changes in the environment (e.g. daytime to promote wakefulness, feeding, and motor activity, or nighttime to promote sleep) (Schibler & Sassone-Corsi, 2002; Kalsbeck et al, 2006). While the SCN can maintain a near 24h oscillation in firing activity when removed from the hypothalamus and placed in culture, removal of peripheral clocks from SCN communication will dampen their rhythms, further demonstrating the SCN's role as the master clock (Reppert & Weaver, 2002).

At a molecular level, the circadian system maintains the near 24h rhythm via both positive and negative transcriptional-translational feedback loops (Ko & Takahashi, 2006). In mammals, two clock genes from the transcription factor family form the positive feedback loop, which in turn drives the negative feedback loop. During the biological day, CLOCK (Circadian Locomotor Output Cycle Kaput) and BMAL1 (Brain Muscle Arnt Like factor) heterodimerize and bind to the E-box promoter sequence (CACGTG), triggering the transcription of both Period

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(Per1, Per2, and Per3) and Cryptochrome (Cry1 and Cry2) genes (Young & Kay, 2001; Reppert & Weaver, 2002; Lowrey & Takahashi, 2004; Ko & Takahashi, 2006). The resultant PER and CRY proteins accumulate and dimerize in the cytoplasm and translocate back into the nucleus at night where the proteins inhibit the action of the CLOCK-BMAL1 complex, thereby completing the negative limb of the feedback loop by inhibiting their own transcription (Kume et al, 1999; Vitaterna et al, 1999). Post-transcriptional phosphorylation of PER and CRY by casein kinase slows the translocation of the proteins to the nucleus and is vital to the proper timing of the system (Lowrey et al, 2000). The CLOCK-BMAL1 complex also initiates transcription of orphan nuclear receptors reverse erythroblastosis virus α (Rev-erbα) and retinoic acid receptor-related orphan receptor α (Rorα) which inhibit and activate BMAL1 expression respectively (Guillaumond et al, 2005). During the biological night, the PER and CRY proteins are degraded, thereby freeing the CLOCK-BMAL1 complex to again influence transcription, making the transcriptional-translation feedback loop complete around 24h later (Takahashi et al, 2008).

Markers of the Circadian System

The near 24h oscillation of the SCN cannot be directly measured in humans, and therefore the neuronal and humoral outputs of the SCN are used to measure the human circadian rhythm. The period (time it takes for an oscillation to complete a full cycle) and phase (time between a circadian reference time point with a different circadian reference point) of these neuronal and humoral markers are measurements used to describe the timing of the circadian clock. The hormones melatonin and cortisol, released from the pineal and adrenal glands,

respectively, and core body temperature (CBT) are commonly used markers for the internal clock.

Melatonin release is mediated by the SCN. This pathway involves neuronal projections from the SCN to the paraventricular nucleus (PVN) of the hypothalamus, that then signal the intermediolateral cell column of the spinal cord to trigger projections to the superior cervical ganglion. Finally, the superior cervical ganglion sends signals to the pineal gland where melatonin is released (Teclemariam-Mesbah et al, 1999). In humans, melatonin levels rise prior to sleep onset with peak levels during the middle of the night and a subsequent decrease in the morning.

Cortisol, a hormone commonly used as a marker of stress in the body, is released from the adrenal cortex and rises during the night with a peak prior to awakening (Hellman et al, 1970; Weitzman et al, 1971). This morning rise in cortisol levels is thought to prepare the body for wakefulness activities such as feeding and locomotion (Wilhelm et al, 2007).

CBT, a marker of metabolic activity, has long been established to display circadian variation. The first published description of oscillations in CBT levels used oral temperature measurements and appeared in a thesis over 170 years ago (Gierse 1842). A half-century later, studies by Pembrey and Nicol further described the CBT trough during the night and peak during the day in humans (Pembrey & Nicol, 1898). Using these oscillations, we can characterize changes in circadian phase and measure the timing of the internal clock.

Exogenous Influences

In order for the endogenous near 24h rhythm to prepare an organism for the environment in which it is living, it must be able to adapt its clock to specific environmental conditions, such as changing day length. Further, the average circadian period in humans is longer than 24h (approximately 24.15h) and thus must be synced or entrained by exogenous stimuli to adapt to the earth's 24h light-dark cycle (Czeisler et al, 1999; Duffy et al, 2011). Both photic and non-photic stimuli may be able to influence the circadian system but, in humans, light is the primary *zeitgeiber* (German for "time-giver") of the circadian system and syncs physiology with the biological day.

Intrinsically photosensitive retinal ganglion cells containing the photopigment melanopsin detect light and transmit this photic information through direct projections to the SCN via the retinohypothalamic tract (Gooley et al, 2001; Berson et al, 2002; Hattar et al, 2002; Reppert & Weaver, 2002). The response of the circadian timing system to light differs depending on the timing of exposure. Photic input in the early portion of the biological night to the SCN will cause the clock to shift to a later time (phase delay). If exposure to photic input occurs in the early biological morning, the clock will shift earlier (phase advance). Furthermore, exposure to light apart from these phase shifting times can have acute effects on the SCN and immediately suppress endogenous melatonin secretion (Lewy 1980).

As with light, temperature has also been observed to entrain the circadian clock. Using extracted pineal glands from chickens, Barrett and colleagues observed temperature compensation of the pineal cells when exposed to constantly increasing temperatures since the melatonin rhythm persisted regardless of the temperature (though a slightly longer period and increased melatonin output were observed for higher temperatures) (Barrett & Takahashi, 1995). Furthermore, when the pineal gland was exposed to temperature pulses at times sensitive to the

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phase shifting effects of light, phase shifting of melatonin release occurred dependent of the timing of the pulse (i.e. temperature pulses in the early biological night phase delayed the clock, and pulses in the early biological morning phase advanced the clock); continued exposure to pulses at these times entrained the circadian rhythm (Barrett & Takahashi, 1995). *In vitro* studies using rat SCN (Ruby et al, 1999) and peroxiredoxin (O'Neill & Reddy, 2011) show similar results. Additionally, changes in ambient temperature can entrain peripheral clocks *in vivo* (Brown et al, 2002).

Studies using animal models have found that feeding can also influence circadian rhythms. The circadian system promotes feeding behavior during the biological day when food is available and physiology is optimal for energy consumption. However, if food is not obtainable during the biological day, animals will entrain to when food is available (Richter 1922). In rats with lesions of the SCN, *ad libitum* feeding schedules result in arrhythmicity of activity cycles, whereas shifted feeding schedules of 12h result in entrainment to the feeding schedule (Stephan et al, 1979). Furthermore, rats with an intact SCN subjected to a 4h restricted feeding protocol during the light period maintained their near 24h rhythmicity within the SCN but liver oscillators entrained to the feeding period, creating a misalignment between central and peripheral clocks (Stokkan et al, 2001). This uncoupling of the central circadian pacemaker from the peripheral clocks has also been observed in other peripheral tissues such as the kidneys and pancreas (Damiola et al, 2000; Pendergast et al, 2013).

Food composition has also been observed to alter circadian rhythmicity. Mice fed with a high fat diet showed a lengthened circadian period (Kohsaka et al, 2007). In humans, individuals who work overnight shifts and consume meals during their biological night often complain about

gastrointestinal disorders (Knutsson 2003), but what happens to their circadian period after eating at these adverse biological times remains unknown and needs to be further explored.

Circadian Protocols

Due to the many types of exogenous stimuli that can influence circadian rhythms in humans, proper controls must be implemented in order to accurately measure these rhythms experimentally. Therefore, researchers have developed several tightly controlled protocols to reduce behavioral and environmental influences upon both the physiology and markers of the circadian rhythm. In a constant routine protocol, subjects are kept awake in constant conditions for an extended period of time to ensure that experimental outcomes are "unmasked" and primarily influenced by the endogenous circadian cycling (Duffy & Wright, 2005). Common constant conditions in human studies include: a dimly lit environment free of time cues to eliminate the alerting and phase shifting effects of light (Wright et al, 1997; Cajochen 2007); remaining sedentary in a constant posture to control for activity and postural changes on physiology (Deacon & Arendt, 1994); an ambient temperature within the thermoneutral range (22-24° Celsius) so as not to impact thermoregulatory physiology; hourly isocaloric meals to spread the intake of food across the 24h day and reduce the influence of feeding; and maintenance of wakefulness for one full circadian cycle to disassociate physiological impact of switching between sleep and wakefulness states (White et al, 1985; Somers et al, 1993; Kräuchi & Wirz-Justice, 2001).

Although a constant routine condition allows researchers to observe changes in circadian physiology, it also contains a major shortcoming in that a sleep debt accumulates throughout the

protocol and resulting fluctuations in performance and behavior cannot be distinguished as being circadian or an effect of sleep deprivation. To disassociate the influences of the sleep and circadian system, researcher Nathanial Kleitman developed what is now known as a forced desynchrony (FD) protocol. In a FD protocol, subjects are kept in constant conditions as described above, but live over a prolonged period of time at a "day" length that is not possible for the circadian system to entrain to (e.g. 20h or 28h) (Czeisler et al, 1999). This allows the subject to sleep and enables the researcher to observe physiologic and behavioral outcomes across the entire circadian period with minimal accumulation of sleep debt, thus disassociating the effects of the sleep and circadian system (Czeisler et al, 1999).

A third protocol employed to study circadian rhythms is an acute reversal of sleep and wakefulness. While adhering to most constant routine conditions, researchers have subjects sleep during their biological day and perform wakefulness activities during their biological night (Weibel et al, 1995; Simon et al, 1998). If the regular cycling of biological or behavioral outcomes persist regardless of wakefulness or sleep states, then these outcomes are considered circadian. If these biological or behavioral rhythms invert, the outcomes are influenced more by sleep and wakefulness states (Brandenberger et al, 1994). The reversal of sleep and wakefulness protocol enables researchers to observe acute relationships between the sleep and circadian system and is a useful tool for examining populations that must rapidly shift their schedules (e.g. night shift workers).

Sleep and Wakefulness

Both sleep and wakefulness have distinct phenotypes that can be characterized by both behavior and brain electrical activity. Sleep is not a single state, but rather a series of several different neurophysiological states or stages throughout the night. The first recognition of different sleep stages came in 1937 when Alfred Loomis and his research group described five different states of sleep using electroencephalography (EEG), noticing differences in the EEG waveform amplitudes and frequencies throughout the night (Loomis et al. 1937). These groundbreaking observations have since been followed by further investigation into, and classification of, the sleep stages using standardized criteria. Separated into either non-REM (NREM) or REM sleep (Carskadon & Dement, 1994), five different sleep stages (NREM Stage 1, NREM Stage 2, NREM Stage 3, NREM Stage 4, and REM) are identified by morphology, frequency, and amplitude (voltage) of the EEG waveforms (Rechtschaffen & Kales, 1968). NREM Stage 1 is the transitional stage between wakefulness and other sleep stages and is the lightest stage of sleep. NREM Stage 2 sleep is characterized by two morphological events known as K-complexes (high voltage, slow frequency) and sleep spindles (bursts of EEG activity). The two slowest frequency stages of sleep are NREM Stage 3 and NREM Stage 4, and collectively they are referred to as slow wave sleep (SWS). Both SWS stages are defined by a high voltage of EEG in the slowest frequency range, with slow waves comprising 20-50% of the EEG signal in Stage 3 and >50% of the EEG signal in Stage 4. It is hypothesized that SWS is a homeostatic marker for sleep pressure based on observations of an increase in time spent in SWS with time awake (Blake & Gerard, 1937; Webb & Agnew, 1971; Dijk et al, 1987). Likewise, sleep during the biological day via naps will reduce SWS during a nighttime sleep episode (Webb & Agnew, 1971; Dijk et al, 1987). REM sleep is characterized by low voltage EEG with mixed frequencies,

sporadic bursts of rapid eye movements, loss of muscle tone, and erratic breathing and heart rate. On average, it takes approximately 90 minutes to cycle through these five stages of sleep (Feinberg & Floyd, 1979).

Sleep Homeostasis and the Circadian System: A Two-Process Model

Sleep and wakefulness are either promoted or inhibited by an interaction of both the homeostatic sleep drive and the circadian system. In 1982, Alexander Borbély described this integration of both the circadian system and sleep homeostasis as the "Two-Process Model of Sleep Regulation" (Borbély, 1982). During the biological day, the homeostatic sleep process builds linearly while the circadian system acts as an opponent process to promote wakefulness. During the biological night, these two systems work in concert to promote a consolidated sleep period (Borbély, 1982; Borbély & Achermann, 1992; Dijk & Czeisler, 1994). The alignment of these two systems in this manner promotes sleep and wakefulness at optimal times, since a desynchrony between these two systems has been shown to disrupt the quality of sleep and wakefulness (Torsvall et al, 1989; Åkerstedt, 2003; Åkerstedt & Wright, 2009), and may result in a circadian misalignment that leads to adverse health (Scheer et al, 2009) and performance outcomes (Wright et al, 2006).

Circadian Misalignment

Circadian misalignment occurs when actions such as physical activity, eating, wakefulness, and sleep occur at adverse circadian times. Social jetlag (interference of social obligations with preferred timing of sleep and wakefulness) (Wittman et al, 2006), travelling jetlag (rapid travel across time zones) (Waterhouse et al, 2003; Waterhouse et al, 2005), and both overnight and early morning shiftwork (Sack et al, 1992; Dumont et al, 2001) are forms of circadian misalignment and have been associated with negative cognitive (Wright et al, 2002; Wright et al, 2006; Markwald & Wright, 2012), health (Knutson et al, 2007; Scheer et al, 2009; Nguyen & Wright, 2009; Buxton et al, 2012; Markwald & Wright, 2012), and sleep outcomes (Torsvall et al, 1989; Åkerstedt, 2003; Åkerstedt & Wright, 2009). As previously stated, the circadian system promotes sleep during the biological night when CBT is low and melatonin concentrations are high. When sleep is initiated at a time when CBT is elevated and melatonin is low, sleep is disturbed and excessive sleepiness is observed during wakefulness (Torsvall et al, 1989; Czeisler et al, 2005; Wright et al, 2006; Åkerstedt & Wright, 2009; Markwald et al, 2010). The types of sleep disruption observed during daytime-initiated sleep episodes include increases of sleep onset latency (SOL) (Åkerstedt, 1990) and reductions of total sleep time (TST), Stage 2, and REM sleep (Torsvall et al, 1989). Mechanisms involving metabolism, sleep, and circadian misalignment in association with negative health outcomes will be discussed later in this review.

Energy and Metabolism

The conversion of energy to work is a fundamental process in biology and necessary to support life function in organisms. The first law of thermodynamics states that energy can neither be created nor destroyed, but rather converted from some other source. Living organisms

can convert energy from the environment in two ways: either through the use of sunlight and photosynthesis (phototrophs), or by the oxidation of foodstuffs and respiration (chemotrophs) (Berg et al 2007). Animals are chemotrophs and thus must consume phototrophs or other chemotrophs in order to obtain energy. This transfer of energy from the phototroph to the chemotroph is done through a series of chemical reactions that either transforms the ingested molecule into another molecule to be used for energy or converts the molecule into a larger biomolecule (Berg et al 2007, Silverthorn 2007, Alberts 2004). This series of chemical reactions in animals, henceforth referred to as metabolism, produces the high-energy cellular intermediate adenosine triphosphate (ATP) to perform work (Erecifiska & Wilson, 1978; Brooks et al 2005). ATP is essential for cellular and skeletal work and is therefore homeostatically maintained throughout the body via fat stores and the promotion of feeding behaviors (Edholm, 1977; Harris et al, 1986; Sims & Danforth, 1987; Horton et al, 1995; Stubbs, 1998).

Energy Balance Hypothesis

The homeostatic maintenance of energy is hypothesized to play a major role in body weight conservation. The energy balance hypothesis states that if energy intake chronically exceeds energy expenditure, energy will be stored as fats and weight gain will occur (Hill & Melanson, 1999). In contrast, if energy expenditure exceeds energy intake, fats will be used for energy and weight will be lost (Spiegelman & Flier, 2001). The internal drive for caloric intake is tightly regulated by anabolic (promoting food intake and storage) and catabolic (promoting decreased food intake and less storage) neuronal systems within the hypothalamus (Woods et al, 1998).

Components of Energy Expenditure

      Daily energy expenditure is composed of four main components: basal metabolic rate (BMR), the thermic effect of food (TEF), exercise activity thermogenesis (EAT), and non-exercise activity thermogenesis (NEAT). BMR accounts for ~60-70% of total daily energy expenditure and is the energy needed to sustain basic physiological functions for vital organs and activities such as breathing, heart rate, and thermoregulation. As BMR must be measured under very controlled conditions with limited sympathetic activity, resting metabolic rate (RMR) is often used instead and is within ~10% accuracy (Levine, 2005). The TEF, accounting for ~10% of the total daily energy expenditure, refers to the rise in temperature after a meal and is the energy needed for the absorption, digestion, and storage of the meal (Miller et al, 1967). EAT and NEAT are the two components of activity thermogenesis and make up the remaining daily energy expenditure. EAT is the energy expended during a purposeful sporting exercise and can range from a negligible influence on daily energy expenditure to as much as ~10% (Levine, 2005). NEAT is the energy expended during the physical activity of daily living (e.g. standing, walking, fidgeting, talking, etc.) and can vary in contribution to total daily energy expenditure from as low as ~15% to as high as ~50% (Levine, 2004; Levine, 2005). Taken together, these four energy-expending activities comprise one limb of the energy balance hypothesis. The other limb is made up of energy intake and it is regulated by central and peripheral signals.

Central Regulation of Energy Homeostasis

Located in the arcuate nucleus (ARC) of the hypothalamus, neuropeptide Y (NPY) neurons release NPY that acts on the PVN and lateral hypothalamic area (LHA) along with agouti gene-related protein (AgRP) to promote feeding and fat storage (i.e. positive energy balance) when fat stores are at a depleted level or during fasting conditions (O'Donohue et al, 1985; Woods et al, 1998; Schwartz et al, 2000; Williams et al, 2001). NPY release is inhibited by a negative feedback loop from peripheral feeding hormones leptin and insulin (Williams et al, 2001), with the PVN acting as the main integration site for energy homeostasis (O'Donohue et al, 1985). Furthermore, injection of NPY directly to the PVN in mice creates a state of positive energy balance by increasing the drive for food intake (Stanley et al, 1986), decreasing energy expenditure (Billington et al, 1991), and altering feedback to white and brown adipose tissue, thus promoting fat storage (Billington et al, 1994). A continued injection of NPY for several days in mice increases body weight and overrides weight maintenance signals (Stanley et al, 1986; Zarjevski et al, 1993). Additionally, NPY exhibits both an ultradian (i.e. periods shorter than 24h that recur throughout the day) rhythm and circadian rhythm in fed conditions (Green et al, 2008). NPY receptors have been identified on the SCN, serving as a potential connection between feeding, fat storage, and shifting of the circadian clock (Card & Moore, 1988; Gribkoff et al, 1998). The LHA, or "feeding center", houses two other neuropeptides that stimulate food intake; melanin-concentrating hormone (MCH) (Rossi et al, 1997) and the wakefulness promoting orexin/hypocretin peptides (de Lecea et al, 1998; Sakurai et al, 1998). Expression of these two neuropeptide systems is up-regulated during fasting and hypoglycemia (Sakurai et al, 1998), though they appear to be weaker stimulators of food intake than NPY expression (Edwards et al, 1999) and do not result in obesity after continued exposure (Williams et al, 2001). As noted previously, NPY receptors are abundant in the LHA, but hypocretin/orexin

peptides can also feedback to the NPY neurons and promote NPY release, providing further evidence that these orexigenic peptides work in concert to promote feeding (Horvath et al, 1999).

If the LHA is the central "feeding center", then the ventromedial hypothalamic nucleus (VMH) is the central "satiety center". Electrical stimulation to the VMH produces inhibition of feeding (Stellar, 1994), while lesioning this brain site is followed by observations of overeating by almost twice the normal meal size and, in mice, a loss of a time of day preference to eat (i.e. mice ate equally during the biological night and the biological day) (Becker & Kissileff, 1971). The VMH is stimulated by circulating leptin and has direct neuronal connections with the PVN and LHA (Williams et al, 2001). Furthermore, melanocortins (MC) (peptides cleaved from the pro-opiomelanocortin polypeptide neurons (POMC) in the ARC are also intricately involved in the central inhibition of feeding. Binding of MC to melanocortin receptors 3 and 4 (MC3-R and MC4-R) result in a decreased drive for feeding and subsequent weight loss (Cone, 1999). Additionally, POMC expression and subsequent MC formation have been observed to act in opposite of NPY neuronal expression (Schwartz et al, 2000) and administration of MC agonists inhibit feeding while antagonists such as AgRP promote feeding (Fan et al, 1997). These inhibitory peptides are also stimulated by leptin via leptin receptors located on the POMC (Seeley et al, 1997) and deficiency of the MC4-R can lead to obesity in mice (Woods et al, 1998).

Peripheral Regulation of Energy Homeostasis

The central nervous system continually receives feedback from peripheral hormones to regulate energy homeostasis by either promoting food intake or satiety. Peripheral satiety signals

include leptin, insulin, peptide YY (PYY), glucagon-like-peptide (GLP-1), and cholecystokinin (CCK), while the primary peripheral feeding signal is the hormone ghrelin.

Leptin has been previously mentioned to interact with the central control of feeding through receptors located on the ARC nucleus, PVN, and lateral hypothalamus. Discovered as the protein transcribed from the obese gene in mutant *ob/ob* mice (Zhang et al, 1994), leptin is produced by white adipose tissue with levels increasing in response to a meal and declining in a fasted state. Circulating levels of leptin are directly correlated to the percentage of fat mass, potentially acting to prevent further weight gain and obesity (Considine et al, 1996). Deficiencies in leptin result in obesity in both mice (Zhang et al, 1994) and humans (Montague et al, 1997). Both central and peripheral administration of leptin decreases feeding (Friedman & Halaas, 1998), as leptin can cross the blood brain barrier and bind to receptors in the ARC nucleus (whereby inhibiting NPY and promoting POMC expression), PVN, and LH, all involved in inhibiting food intake (Baskin et al, 1999). Leptin has been observed to follow a diurnal rhythm under fed conditions with highest levels during the night and lowest in the mid-afternoon (Sinha et al, 1996), but is also influenced by timing of meals (Schoeller et al, 1997) and sleep (Simon et al, 1998). Leptin receptors are also located on the SCN (Håkansson et al, 1998), suggesting that leptin can influence the circadian system. Furthermore, observation of peripheral clocks in white adipose tissue (Zvonic et al, 2006; Gómez-Abellán & Garaulet, 2013) and projections from the SCN to white adipose tissue may suggest a circadian synchronization or influence of leptin (Bartness et al, 2001), and therefore potential mechanisms for adverse consequences during circadian misalignment.

Insulin is a hormone produced acutely by the pancreatic beta cells in response to a glucose challenge and facilitates glucose uptake from the blood into cells with insulin receptors

(e.g. muscle, adipose tissue, liver) while also acting as a satiety hormone. Central administration of insulin acts similarly to leptin by acting on the ARC nucleus to inhibit NPY and POMC expression (Benoit et al, 2004). The transport of insulin to the hypothalamus is not a rapid process and is therefore considered a long-term regulator of body adiposity rather than a short-term satiety signal (Havel, 2001). Furthermore, both leptin and insulin are considered adiposity signals as the intensities of each response are dependent on the proportion of fat mass and both decrease feeding (Woods & Seeley, 2000). Under constant routine conditions, insulin follows a circadian rhythm with a peak in the early biological morning (Morgan et al, 1998, Shea et al, 2005), though this has not yet been observed using a FD protocol (Scheer et al, 2009).

Peptides produced in the gut can promote either satiety or feeding signals. The hormones CCK, PYY, and GLP-1 all inhibit feeding, but the direct pathways can be complicated as both central and peripheral administration both lead to decreases in feeding (Havel, 2001). The first peptide from the gut discovered to promote satiety was CCK. Hypothesized to control meal size and duration, CCK is a short-term inhibitor of food intake with peak levels observed 30min after meal ingestion (Moran, 2000). CCK works synergistically with leptin via the brainstem and hypothalamus for long-term influences on energy balance (Matson et al, 1997). PYY is produced by the small and large intestines and released in response to a meal (Neary et al, 2004), with laboratory-simulated postprandial levels reducing food intake by 30% in lean (Batterham et al, 2002) and obese (Batterham et al, 2003) individuals. Binding to the Y2 receptor in the hypothalamus, PYY decreases NPY mRNA, inhibits NPY nerve activation, and increases POMC neuronal activity thus inhibiting the drive for feeding (Batterham et al, 2002). Stimulation of this pathway may be a possible therapeutic target for treatment of obesity, as obese individuals have a noted desensitivity to leptin and insulin (Batterham et al, 2003). GLP-1 is secreted alongside

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PYY in the small and large intestine and enhances insulin secretion after a meal (Kreymann et al, 1987). These increases in insulin secretion have also been observed after GLP-1 administration in the absence of a meal (Kreymann et al, 1987). Central and peripheral GLP-1 administration decreases energy intake (Turton et al, 1996; Verdich et al, 2001) with increased neuronal activity in the PVN (Turton 1996). CKK, PYY, and GLP-1 have not been identified to have a circadian rhythm in their productions (Scheer et al, 2013).

Ghrelin is produced from the stomach and is the only peripheral hormone known to stimulate feeding (Neary et al, 2004). Produced during fasting conditions and reduced when nutrients are available (Havel, 2001), ghrelin acts centrally on the hypothalamus to excite both NPY and AgRP neuronal expression (Shintani et al, 2001; Chen et al, 2004) and excite the orexin/hypocretin system (Toshinai et al, 2003) to promote feeding. In humans, intravenous infusion of ghrelin during an "all you can eat" buffet led to a 28% increase in food intake as compared to saline controls (Wren et al, 2001). Continued exposure to ghrelin in rodents results in increased carbohydrate utilization accompanied with decreased fat oxidation, leading to increased body fat (Tschöp et al, 2000). Ghrelin appears to follow a diurnal rhythm with increases in the early portion of the night and decreases in the second half of the night with a trough in the morning at ~08:00 (Bodosi et al, 2004; Dzaja et al, 2004; Espelund et al, 2005) These rhythms are blunted with sleep restriction which suggests that ghrelin is influenced by both the circadian and the sleep and wakefulness systems (Bodosi et al, 2004; Dzaja et al, 2004).

The Circadian System, Sleep, and Energy: Fundamental Integrations

The circadian system and the body's energy state are intimately coupled together. In simple single celled cyanobacteria, production of ATP via the phosphorylation of ADP follows a circadian rhythm that can be entrained by the light-dark cycle (Rust et al, 2011). Furthermore, subjecting these cyanobacteria to circadian misalignment of an 8h dark period during the biological day followed by a light pulse reduced the ATP/ADP ratio and in turn reduced enzymatic reactions important both for ATP metabolism and circadian regulation, indicating a coupling of the clock and metabolism (Rust et al, 2011). In red blood cells that are dependent solely on glycolysis for ATP production, assayed NADH/NADPH ratios, and therefore ATP production, also exhibit a clear ~24h circadian rhythm (O'Neill & Reddy, 2011). In more complex systems, the circadian system and metabolism are linked at a genetic level. Gene expression in muscle, both white and brown adipose tissue, and the liver have all been identified to follow a circadian rhythm in expression with the identified rhythmic genes playing key roles in oxidative phosphorylation and fat metabolism (Yang et al, 2006; Green et al, 2008). Additionally, the rate-limiting enzymes in many of these metabolic processes follow circadian rhythms (Panda et al, 2002). At a transcriptional level, the CLOCK-BMAL1 complex appears to be a major participant in the integration of the circadian system and metabolism. Mice with a mutation in CLOCK not only show an altered and blunted circadian rhythm in wheel running in comparison with heterozygous mice, but also exhibit a decrease in energy expenditure with an increase in caloric intake and body mass (Turek et al, 2005). Orphan nuclear hormone receptors Rev-erbα and Rorα, which are critical for regulation of BMAL1 expression in the circadian transcriptional-translational feedback loop, are also vital for regulation of lipid metabolism (Fontaine & Staels, 2007). Dysregulation of BMAL-1 in mice leads to changes in energy and

lipid homeostasis similar to that of metabolic syndrome (Shimba et al, 2011). Furthermore, Rev-erbα and Rev-erbβ double knockout mice show both circadian arrythmicity in wheel running activity and a deregulation of lipid metabolism (Cho et al, 2012). Similarly, PGC-1α, the transcriptional co-activator that regulates nuclear hormone receptors, has been suggested to be the key integrator of clock and metabolic pathways since it has a robust circadian rhythm and is susceptible to environmental and nutritional signals (Lin et al, 2008).

Little is known about the integration between whole body energy expenditure and the circadian system in humans since the evidence for a circadian rhythm in resting metabolism is inconclusive. Using a 41h constant routine protocol, one group of researchers observed a near 24h endogenous rhythm in carbon dioxide production with a peak in the evening around 18:00 (Spengler et al, 2000). In men under a 34h constant routine protocol and using heat production as a metric for metabolism, Krauchi and colleagues observed a 24h rhythm to metabolism with a peak during the middle of the day and a trough between midnight and 06:00 (Krauchi et al, 1994). Recent evidence has emerged from a FD protocol indicating that the peak hunger ratings on a visual analog scale are reached at ~20:00 and a trough ~07:50, potentially driving metabolism to prepare for the nightly fast (Scheer et al, 2013). Further studies are needed to clarify where the circadian peak and trough of metabolism may reside and how misalignment may alter this relationship.

Circadian Misalignment and Energy Metabolism

The integration of the circadian system and energy metabolism can be observed through adverse health outcomes associated with the misalignment between caloric intake and the proper

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circadian timing of metabolism. Night shift workers often complain of gastrointestinal disorders (Segawa et al, 1987; Knutsson 2003, Drake et al, 2004) and have an increased risk for obesity (Di Lorenzo et al, 2003; Antunes et al, 2010) along with other health disorders. As previously mentioned, feeding with a high fat diet can disrupt and lengthen the timing of the circadian clock in mice (Kohsaka et al, 2007) and cause desynchronization of peripheral clocks due to this SCN disruption, as peripheral organs entrain to feeding schedules during the biological night (Damiola et al, 2000; Stokken et al, 2001). Allowing mice to eat only during their biological night (daytime) and inducing a circadian misalignment of eating and metabolism increases body mass as compared to mice eating at the correct biological time (nighttime), without a significant difference in caloric intake or locomotor activity (Arble et al, 2009). Similarly, Salgado-Delgado and colleagues found that simulating night shift work in mice shifted feeding patterns to the "working" hours (biological night, daytime) and increased abdominal obesity with no difference in food ingestion. Restricting food intake to the active phase while continuing to maintain a night shift schedule abolished these differences (Salgado-Delgado et al, 2010). Exposing mice to constant bright light throughout the 24h day, thereby causing the internal clock to free run and desynchronize feeding from proper circadian timing, results in increases in body mass with simultaneous reductions in glucose tolerance in the absence of increased caloric intake or decreased activity (Fonken et al, 2010). Subsequent restriction of the feeding opportunity to the correct biological time prevented the weight gain in the mice (Fonken et al, 2010). Studies that subjected mice to either constant bright lighting and normal chow, constant bright lighting and high fat chow, a light-dark cycle and high fat chow, or a light-dark cycle with normal chow revealed that mice in the constant bright light, with both normal and high fat chow, and mice in the light-dark cycle with high fat chow gain weight via increased energy intake and decreased

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energy expenditure (Coomans et al, 2013). Interestingly, mice in the constant bright light with normal chow condition gained weight faster than the mice that were fed the high fat chow in the light-dark cycle. The group eating the high fat chow under constant bright light exhibited an additive effect (Coomans et al, 2013).

In humans, eating during the biological night results in a decreased TEF as compared to eating the same meal during the biological day (Romon et al, 1993). This finding could represent a potential mechanism as to why the rodents eating during the biological night gain weight in the absence of caloric intake or energy expenditure differences. While decreases in RMR or TEF are not observed when comparing morning caloric intake to afternoon intake (Weststrate et al, 1989), eating an "early" lunch (before 15:00) rather than a "late" lunch (after 15:00) has been associated with greater weight loss during active dieting with no differences between the two groups energy expenditure, caloric intake, or sleep duration (Garaulet et al, 2013). Subjects in the "late" lunch group were also more likely to skip breakfast and be considered evening types, potentially leading to post lunch meals occurring later in the evening than "early" lunch eaters. Pushing meals into the later evening could be an explanation for the increased risk of obesity in shift workers as caloric intake past 20:00 can predict BMI, if sleep timing and duration are controlled (Baron et al, 2011). Individuals with Night Eating Syndrome, a condition in which persons follow a pattern of consuming food late into the evening and throughout the night, are associated with a higher BMI and binge eating tendencies (Colles et al, 2007). Furthermore, eating snacks during the night has been linked to decreased fat oxidation and increases in total and LDL cholesterol in women (Hibi et al, 2013).

Composition of diet, eaten chronically, could also shift the clock to promote caloric intake at adverse times. Chronic feeding of high fat diets to mice advances peripheral clocks and

misaligns feeding from circadian promotion of feeding via the SCN (Pendergast et al, 2013). Mendoza and colleagues discovered that mice eating a high fat diet for 3 months were impaired in their response to light entrainment, thus causing a greater "jet lag" during periodic shifts in light (Mendoza et al, 2008). Both studies provide evidence of diet potentially leading to chronic misalignment.

Circadian misalignment also alters the levels of peripheral regulators of energy expenditure, providing potential mechanistic explanations for the adverse health outcomes. Studies simulating circadian misalignment in the laboratory using phase shifting and free running protocols have observed decreased leptin during wakefulness, increased levels of the hormone at night (Scheer et al, 2009; Nguyen et al, 2010), and increased glucose levels despite concurrent increases in insulin (Hampton et al, 1996; Scheer et al, 2009; Buxton et al, 2012; Gonnissen et al, 2012). Levels of ghrelin and GLP-1 were observed to follow the patterns of meal administration during the misalignment, with phase delayed subjects exhibiting a decrease in GLP-1 concentrations (Gonnisen et al, 2012).

Attempting to sleep at a time in which the circadian system is promoting wakefulness, common in circadian misalignment, results in disturbed sleep. To more fully understand how circadian misalignment affects metabolism, measures of energy expenditure and sleep need should also be considered since their integration is vital to healthy physiology.

Sleep and Energy Metabolism

One of the proposed functions of sleep is the conservation of energy during the biological night. In a seminal paper in 1983, Rechschaffen and collaborators demonstrated the importance

of this energy conservation, though unbeknownst to the group at the time. The study used a

rodent model in which two rats were attached to EEG with one mouse being monitored by

researchers and deprived of sleep while the other rat was able to sleep *ad libitum,* acting as a

"yoked" control (Rechtschaffen et al, 1983). To deprive the experimental rat of sleep, researchers

placed each mouse on an opposing side of a rotating disk separated by a wall and situated the

disk above water. When the sleep deprived rat entered sleep as determined via EEG, the disk

would rotate and push the mouse into the water. Since rats have an aversion to water, the rodent

would awaken and climb back onto the disk, thereby depriving the rat of sleep. The yoked

control was able to sleep at any time the disk was not rotating. Due to increased wakefulness, the

sleep deprived rat expended significantly more energy than their yoked control (Bergmann et al,

1989) and several died during the experiment (Rechtschaffen et al, 1983). Though food and

water were available *ad libitum*, the sleep-deprived rat began to adopt a malnutrition phenotype

and lost the ability to thermoregulate. This suggests that the rat could not consume enough

energy to meet the additional energy requirements from sleep deprivation and maintain its body

temperature (Bergmann et al, 1989). Follow up studies in humans have determined that energy

expenditure is lower during sleep than during wakefulness, as observed through decreased

sleeping metabolic rate (Kreider et al, 1958; Fraser et al, 1989; Bonnet et al, 1991) and increased

energy expenditure in subjects during controlled constant routine conditions with 24h total sleep

deprivation (Jung et al, 2011). During sleep deprivation, energy expenditure is ~32% higher than

during a sleeping opportunity and ~7% higher over an entire 24h period (Jung et al, 2011).

Following the sleep deprivation, a recovery sleep episode exhibited significantly lower energy

expenditure than a baseline night of sleep, furthering the notion of an energy balance fluctuating

around a homeostatically regulated set point (Jung et al, 2011). Reduced energy expenditure

(~5%) and TEF (~20%) have also been reported the morning following a night of total sleep deprivation (Benedict et al, 2011).

Though total sleep deprivation clearly demonstrates increased energy demands as opposed to sleeping, the energy costs of disturbed and restricted sleep should also be considered since they commonly occur in modern society. Fragmented sleep induced by acoustic stimulation during the biological night can result in increased energy expenditure concurrent with decreases in REM and SWS and increases in the lighter Stage 1 sleep (Bonnet et al, 1991). The amount of SWS has been inversely correlated with energy balance while REM sleep has been positively correlated with energy balance (Rutters et al, 2012), though findings comparing energy expenditure levels during different sleep stages have been inconsistent. In a single-blind cross-over design experiment in which subjects were forced to turn off an alarm intermittently while sleeping in a respiratory chamber, fragmented sleep not only led to decreases in TST, SWS, and REM sleep, but also was associated with increases in carbohydrate oxidation, EAT, exhaustion, and decreases in fat oxidation (Hursel et al, 2011).

Insufficient Sleep and Energy Metabolism

Due to demands of modern society with work, school, and social schedules, insufficient sleep is a common report amongst both adults (Krueger et al, 2009) and adolescents (Carskadon, 2011). As mentioned previously, shift workers tend to have both disturbed and insufficient sleep schedules (Torsvall et al, 1989). Numerous associations of restricted sleep and obesity have been reported (Patel & Hu, 2008; Bo et al, 2011; Mozaffarian et al, 2011), with potential mechanisms now being revealed from laboratory studies. These reports of weight gain due to sleep restriction

alone do not comply with the energy balance hypothesis, as the increased amount of wakefulness and associated increase in energy expenditure would lead to a negative shift in energy balance and consequent weight loss. However, restricting sleep in the laboratory pushes subjects towards positive energy balance via increased food intake when access to food is *ad libitum* (Brondel et al, 2010; Markwald et al, 2013). During sleep restriction, subjects have reported increased hunger and appetite on visual analog scales (Spiegel et al, 2004b; Schmid et al, 2008; Nedeltcheva et al, 2010) and fMRI scans have revealed increased activation of brain regions that may be sensitive to food stimulation (St. Onge et al, 2012). One possible mechanistic explanation for increased caloric intake is alterations in the peripheral feeding hormones. Acute sleep restriction and controlled caloric intake lowers maximal and rhythmic 24h leptin (Spiegel et al, 2004a; Spiegel et al, 2004b; Taheri et al, 2004; Chaput et al, 2007), insulin sensitivity (Spiegel et al, 2005; Buxton et al, 2010), insulin concentrations (Nedeltcheva et al, 2010), and signaling to adipose tissue (Broussard et al, 2012), but increases circulating ghrelin levels (Taheri et al, 2004; Spiegel et al, 2004b; Schmid et al, 2008), all promoting a state of positive energy balance via caloric intake. Combining sleep restriction with circadian misalignment using a FD protocol, researchers found increases in glucose concentrations in response to meals, due to insufficient insulin production during the sleep restricted periods; the metabolic rate was also decreased (Buxton et al, 2012). Interestingly, these responses stabilized back to baseline levels after a 9 day recovery period (Buxton et al, 2012). In overweight individuals participating in a caloric restriction protocol, sleep restriction reduced fat oxidation and thereby decreased the amount of weight lost as fat by 55%, and increased weight loss via fat free body mass by 60% (Nedeltcheva et al, 2010).

Sleep restriction may also promote eating at an adverse circadian time for food consumption. In a 14-15 day randomized cross-over design study in which subjects were either given a 5h or 9h sleep opportunity for 5 days with *ad libitum* food access, Markwald and colleagues found that subjects in the sleep restricted 5h group not only gained 0.82 kg on average despite expending ~5% more energy than the 9h condition, but also consumed more calories in post dinner snacks than any other individual meal (Markwald et al, 2013). These findings indicate that subjects in a sleep-deprived state eat far more than the additional energy demands required to maintain wakefulness (Markwald et al, 2013). Furthermore, Nedeltcheva and colleagues found that restricting subjects to 5.5h of sleep as opposed to 8h of sleep resulted in increased snack consumption between the hours of 19:00-07:00 (Nedeltcheva et al, 2009). The interactions of sleeping during the biological day, with the inherent sleep disruption and restriction, and consumption of the majority of calories during the biological night with energy balance, peripheral hormone regulation, and substrate oxidation in humans are unknown and need to be further explored.

Temperature, Sleep, and Energy Metabolism

The byproduct of energy metabolism is heat, which is produced by the core via the regular functioning and metabolism of the internal organs (e.g. heart, liver, kidneys, and brain). This heat production is homeostatically maintained around a thermoregulatory set point and controlled by neurons in the hypothalamus (Benzinger, 1969; Mekjavic & Eiken, 2006; Kräuchi, 2007). This maintenance, or thermoregulation, is vital for enabling endotherms to live in a multitude of climates. The preoptic anterior hypothalamus (POAH) is primarily responsible for

thermoregulation and can facilitate appropriate physiological responses to temperature challenges (e.g. vasodilation/constriction of the vasculature, shivering and panting) (Boulant & Dean, 1986). If POAH temperature exceeds the thermoregulatory set point, heat is dissipated through the periphery by interacting with the environment via convection and radiation, but if temperature falls below the set point, then metabolism is increased to produce heat and the periphery acts as a buffer with the external environment to protect the core temperature from drastic temperature fluctuations (Kräuchi & Wirz-Justice 2001; Boulant, 2006). During both wakefulness and sleep, Glotzbach and Heller found that lowering hypothalamic temperatures in kangaroo rats resulted in increases in metabolic output, but that the increased metabolism was impaired during SWS and almost non-existent during REM (Glotzbach & Heller, 1976). These observations have led to the hypothesis that the thermoregulatory set point during sleep may be lower than during wakefulness and may help to minimize metabolic production needed to maintain thermoregulation, and thereby lowering 24h energy expenditure. Furthermore, the circadian regulation of CBT, as described previously, combined with the sleep-induced decline in CBT (Kräuchi & Wirz-Justice 2001) would require increased metabolic production during the night if the set point were not lowered. This is important because regardless of an organism's temperature preference, most if not all species choose to sleep or rest during the trough of their CBT rhythm (Kräuchi & Wirz-Justice 2001).

Temperature and Promotion of Sleep

POAH activity is integrated with sleep and wakefulness states. Changes in the firing rate of POAH thermosensitive neurons have been identified prior to sleep onset and during

wakefulness. Warm sensitive neurons (WSN) in the sleep promoting centers of the POAH (e.g. the Ventral Lateral Pre-Optic area and Median Pre-Optic areas) increase their firing rates to suppress arousal-related cell types and decrease their firing rates prior to and throughout wakefulness (DeArmond & Fusco, 1971; Alam et al, 1995; Alam et al, 1996; McGinty & Szymusiak, 2001). Contrastingly, cold sensitive neurons appear to be active during wakefulness (McGinty & Szymusiak, 2001). Additionally, Roberts and Robinson observed that locally warming POAH thermoreceptor neurons with implanted diathermic electrodes induced sleep in cats (Roberts & Robinson, 1969) while Szymusiak and colleagues found that POAH cell loss reduced both total and SWS, but heating restored the sleep parameters (Szymusiakl et al, 1991). WSN have also been identified in peripheral skin sites which when heated can relay information to the brain and increase POAH WSN firing rates, thereby promoting sleep (Boulant & Bignell, 1973; Boulant & Hardy, 1974; Van Someren, 2000).

Sleep initiation in humans typically occurs at night when the circadian CBT rhythm declines, with wakefulness occurring throughout the rise of the CBT rhythm in the biological morning (Czeisler et al, 1980; Zulley et al, 1981; Campbell & Broughton, 1994; Kräuchi & Wirz-Justice, 2001). In an observation of elderly subjects, Campbell and Broughton measured CBT and sleep EEG during subjects' habitual sleep times and found that sleep is most likely to occur when CBT is declining at its maximum rate (Campbell & Broughton, 1994). However, prior to the decline in CBT, heat is released to the environment via a shunting of blood to peripheral cutaneous vascular structures that can regulate skin blood flow (i.e. arteriovenous anastomoses [AVAs] and capillaries) (Krogstad et al, 1995). Skin temperature gradients reflect blood flow, therefore measures of peripheral temperature can be used as a marker of heat production in the core (Rubinstein & Sessler, 1990).

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The distal-to-proximal skin temperature gradient (DPG) is an indirect measurement of heat loss in which temperature at distal skin sites (e.g. hands or feet) is subtracted from proximal skin sites (e.g., subclavicular area, head, or stomach). Using the constant routine protocol in men, Kräuchi and Wirz-Justice observed the circadian rhythms of core and peripheral skin temperatures and found that proximal skin temperatures were regulated by capillaries and followed a similar circadian pattern as the CBT rhythm, but the distal skin temperatures were opposite in pattern and increased due to shunting and heat loss through the periphery (Kräuchi & Wirz-Justice, 1994). As blood flows to the periphery via vasodilation and skin temperature warms, the distal site becomes closer in temperature to the proximal site and the DPG becomes narrower. A larger difference between proximal and distal skin temperatures is described as a wider DPG. Prior to habitual sleep time and the decline in CBT, the DPG narrows whereas prior to habitual waketime the DPG widens (Kräuchi & Wirz-Justice, 1994). The change in DPG has been reported to be a better predictor for faster SOL than rate of CBT decline, melatonin onset, or subjective sleepiness ratings (Rubinstein & Sessler, 1990; Smolander et al, 1993; Kräuchi et al, 1997; Kräuchi et al, 1999; Kräuchi et al, 2000). Mechanistically, the rise in DPG triggers the temperature sensitive neurons in the distal skin that will excite the WSN in the hypothalamus to promote sleep (Boulant & Bignell, 1973; Boulant & Hardy, 1974; Lowry et al 2009).

Exogenous Manipulations

The relationship between distal and proximal skin temperatures with sleep onset and architecture can be manipulated by exogenous influences. Kräuchi and colleagues have proposed that the release of melatonin could be the driving factor for vasodilation in vasculature and will

consequently increase peripheral heat loss via increased blood flow to the periphery prior to sleep (Kräuchi et al, 2002). Melatonin acts to initiate a vasodilatory response by binding to the MT-2 receptors in the periphery (preferentially located near or on the AVAs) (Dubcovich & Markowska, 2005). Exogenous melatonin administration during the daytime hours, when both melatonin and peripheral temperatures are low, leads to increases in both sleepiness and peripheral heat gain (Kräuchi et al, 2002). Furthermore, administration of exogenous melatonin prior to the circadian onset of melatonin release results in phase advances of not only melatonin onset, but also the rise in distal temperatures and drop in proximal and CBT (Kräuchi et al, 1997). The administration of the MT-1/MT-2 receptor agonist Ramelteon during daytime hours also leads to decreases in CBT and narrowing in the DPG, supporting the role of melatonin receptors in the mechanisms of thermoregulation (Markwald et al, 2010). Conversely, bright light administration and melatonin suppression have been observed to widen the DPG and subsequently increase CBT (Cajochen et al, 2005). This hypothesized mechanism for melatonin in the periphery lends insight to the circadian thermoregulatory physiology that is observed prior to habitual sleep. Under these assumptions, it is the vasodilation of the periphery that mediates the decline of CBT and not the core's vasoconstriction that is mediating the rise in the DPG (Gradisar & Lack, 2004).

Raymann and colleagues have further manipulated the relationship between skin temperatures and sleep architecture by placing subjects in a temperature-controlled thermosuit during sleep and subtly changing skin temperatures without changing CBT (Raymann et al, 2008). These researchers found that by increasing proximal temperatures by as little as 0.4^OC, sleep shifted to the deeper stages and nightly awakening decreased in both healthy young and older adults (Raymann et al, 2008). Using the same thermosuit, researchers altered proximal and

distal skin temperatures of narcoleptics in a constant routine protocol and found that cooling the

distal skin site increased daytime wakefulness by 24% while warming CBT increased reaction

times by 25% (Fronczek et al, 2008b). Similar to healthy subjects, proximal warming of the

narcoleptics during sleep increased the deeper stages of sleep and decreased wakefulness

(Fronczek et al, 2008a).

Altering the relationship between skin temperature and alertness may be a possible

therapy for individuals needing to sleep at adverse circadian times. Likewise, this relationship

may be beneficial for those working extended shifts at adverse circadian times when alertness is

vital for performance and maintaining vigilance is difficult. It is currently unknown how alerting

agents such as caffeine affect this relationship and needs to be further explored.

Energy Metabolism and Temperature

A clear relationship between temperature and energy metabolism can be identified after

the consumption of a meal during the TEF. During a constant routine protocol, Kräuchi and

colleagues determined that per 159 kcal of food consumed, CBT rises 0.01^O C (Kräuchi et al,

1994). Conversely, rhesus monkeys subjected to a caloric restriction protocol demonstrated a

decrease in CBT during the fasted state (Lane et al, 1996). The rise in CBT after a meal has also

been found to potentially increase blood flow to the periphery and thereby increase distal skin

temperatures (Hirai et al, 1991).

The observed drop in energy expenditure during sleep may be in part due to the decline in

CBT. As reviewed previously, cooling of the hypothalamus is followed by increases in

metabolism in an attempt to maintain temperature around a thermoregulatory set point

(Glotzbach & Heller, 1976). Furthermore, researchers simulating a cold environment in an environmental chamber have found increases in energy expenditure accompanied by declines in both CBT and skin temperatures and a widening of the DPG (van Marken Lichtenbelt et al, 2002). Placing subjects in a warm environmental chamber induced increases in CBT and skin temperatures with a dip in energy expenditure and a narrowing of the DPG (van Marken Lichtenbelt et al, 2001). However, if CBT were the only factor driving reductions in energy, we would observe a circadian rhythm to energy expenditure similar to that of the CBT rhythm. Jung and colleagues did not observe a distinct dip in energy expenditure during the night in a 24h sleep deprivation protocol, though they did not measure CBT to track both metabolism and temperature (Jung et al, 2011).

It is unclear how the natural rhythms of the DPG and energy expenditure interact, but the relationship between energy expenditure and SOL might provide some evidence, since a narrowing of the DPG is the best predictor of SOL. If sleep is obtained at a faster rate, less energy is needed to maintain the wakefulness occurring prior to sleep onset. Further studies are needed to examine the relationship between the DPG and energy expenditure.

Circadian Misalignment and DPG

The effects of circadian misalignment on the DPG are currently unknown. Again, since the DPG is the best predictor for SOL, misaligning sleep from the circadian rhythm may increase wakefulness and therefore increase 24h energy expenditure. Shift workers trying to sleep at adverse circadian times may benefit from manipulations to the DPG such as use of exogenous melatonin or melatonin agonists, but a future study observing the shift in the DPG during

circadian misalignment and simulated overnight shift work is needed to determine such a relationship.

Summary

This review has highlighted the fundamental integration of the circadian system with sleep, energy expenditure, and thermoregulation. Furthermore, this review examined the potential consequences of circadian misalignment with healthy physiology. As weight gain and its associated diseases have become a burden on our society, it is vital to understand the contributions to metabolic syndrome. The circadian system actively promotes wakefulness activities during the biological day and sleep during the biological night in humans and when this system is misaligned due to work, social obligations, or disease states, we begin to see adverse health outcomes such as obesity, cardiovascular disease, insulin resistance, and cancer. Future studies aimed at understanding the fundamental changes in physiology during circadian misalignment are needed in order to recognize potential mechanisms that may produce adverse health outcomes. We can then develop strategies to avoid and counter these adverse health outcomes in individuals that are misaligned.
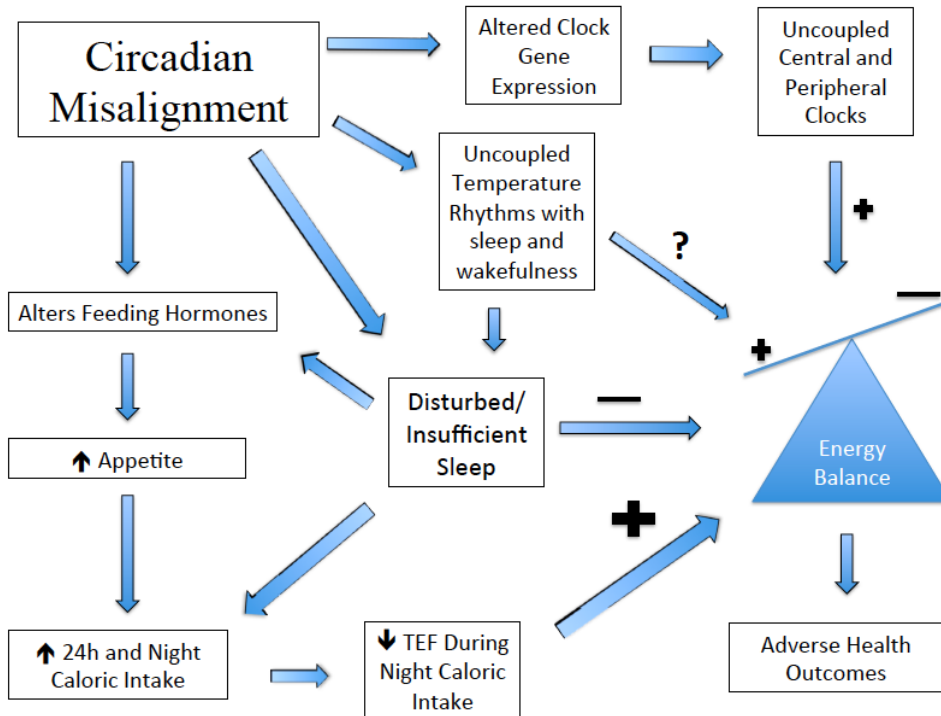
Figure 1. Working model for mechanisms of how circadian misalignment may lead to possible weight gain and adverse health outcomes. Circadian misalignment will alter feeding hormones that will increase appetite and lead to increased caloric intake, notably at times when metabolism is not promoted by the circadian system. Attempting to sleep when the circadian system is promoting wakefulness via activation of temperature and arousal systems, results in disturbed sleep which increases energy expenditure initially, but ultimately leads to caloric intake that is far greater than the expenditure observed at adverse circadian times. Likewise, eating at adverse circadian times may uncouple peripheral and central clocks, thereby promoting positive energy balance. The contribution of each of these potential mechanisms of positive energy balance needs to be further studied in humans.

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CHAPTER 2

**IMPACT OF CIRCADIAN MISALIGNMENT ON ENERGY METABOLISM AND
SLEEP DURING SIMULATED NIGHT SHIFT WORK**

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ABSTRACT

Eating during the biological night, a time in which the internal circadian clock promotes sleep, is a novel risk factor for weight gain and obesity, yet little is known about the mechanisms by which such circadian misalignment leads to metabolic dysregulation in humans. We studied 15 adults in a 6-d inpatient simulated shiftwork protocol and quantified changes in energy expenditure, substrate utilization, appetitive hormones, sleep, and circadian phase during day versus nightshift work. We found that total daily energy expenditure increased by ~4% on the transition day to the first nightshift, which consisted of an afternoon nap and extended wakefulness, whereas total daily energy expenditure decreased by ~3% on the second and third nightshifts, which consisted of afternoon and nighttime wakefulness followed by daytime sleep. Further, energy expenditure was decreased by ~12% during daytime sleep episodes, despite disturbed sleep. Energy expenditure after food consumption was also decreased in response to dinner on the first nightshift. Also, night shiftwork increased total daily fat utilization on the first and second nightshifts and reduced carbohydrate and protein utilization on the second nightshift. Ratings of hunger were decreased during night shiftwork despite concurrent decreases in 24h levels of the satiety hormones leptin and peptide-YY. Findings suggest that reduced energy expenditure during nightshift work and reduced energy expenditure in response to dinner may represent contributing mechanisms by which working and eating during the biological night increases the risk of weight gain and obesity.

INTRODUCTION

Emerging evidence from animal models indicates a fundamental interplay between circadian and metabolic physiology that may have important implications in understanding metabolic health and disease (1, 2). The role of the circadian timekeeping system in coordinating physiological and behavioral events so that they occur at an appropriate environmental time of day is widely recognized (e.g., sleeping during the solar day and activity at night for nocturnal species). Further, disruption of the intrinsic circadian timing system can lead to adverse health consequences (3, 4). Eating at an inappropriate circadian time, when the internal circadian clock is not prepared for food intake (e.g., eating at night), is considered a novel risk factor for weight gain and obesity, yet little research has been conducted in humans on this topic.

The endogenous circadian time keeping system in humans has evolved to modulate energy metabolism so that wakefulness, activity, and meal consumption are promoted during the solar day and that sleep, inactivity, and fasting occur during the solar night (5). With the widespread use of electrical lighting however, work and social activities are capable of being extended further into the night at a time that the internal clock promotes sleep (6, 7). Being awake during the biological night leads to disturbed physiology and behavior as it creates a state of desynchrony between the circadian clock and wakefulness-sleep cycle known as circadian misalignment. Circadian misalignment is common in shiftwork. More than 20% of adults in the United States work non-traditional hours (8) and eat some of their meals during the biological night (9). Further, shiftwork is associated with increased risk for obesity (10), metabolic syndrome (11), cancer (12), and diabetes (13). Additionally, laboratory induced circadian misalignment decreases the satiety hormone leptin (4, 14) and blunts the post meal suppression

of the hunger stimulating hormone ghrelin (15), which could promote hunger, and increase food intake and subsequently lead to weight gain.

Recent evidence from animal models reveal that the consumption of calories during adverse circadian times contributes to weight gain (16-20) and health problems such as decreased insulin sensitivity (18, 19). Further, intake of the same amount of calories during the typical sleep time lead to greater weight gain than when consumed during the typical waketime (16, 18), suggesting that a calorie consumed during the time normally reserved for sleep may be more responsible for weight gain than the same calorie consumed during the time normally reserved for wakefulness. Furthermore, eating snacks during the night has been linked to decreased fat utilization and increased total and LDL cholesterol in women as compared to the same snacks consumed during the day (21). While eating during the biological night appears to be a risk factor for unwanted weight gain, mechanisms underlying the increased risk in humans remain unknown. Therefore, the primary aim of this study was to quantify total daily energy expenditure (EE), the EE after food intake—referred to as the thermic effect of food (TEF), and total daily substrate utilization via whole room indirect calorimetery. A 6-day, inpatient simulated night shiftwork protocol at the University of Colorado Hospital Clinical Translational Research Center (CTRC) that included a controlled research diet designed to meet energy balance at baseline, was used to examine the impact of circadian misalignment on sleep architecture, hunger ratings, and the appetitive hormone ghrelin (increases food intake) and satiety hormones leptin and peptide-YY (PYY reduces food intake). We hypothesized that circadian misalignment would disrupt sleep, reduce fat utilization, reduce TEF following food intake during the biological night, and alter circulating concentrations of feeding hormones associated with increased appetite (reduced leptin (4, 14)and PYY levels and increased ghrelin

levels (4)). We also hypothesized that circadian misalignment would result in higher EE during the daytime sleep episode; associated with greater number and duration of arousals during the daytime sleep opportunity (22).

Results

Circadian Melatonin Rhythm and Sleep. Circadian misalignment occurred during the simulated night shiftwork protocol (Fig. 1) as the circadian melatonin rhythm did not adapt to the nigh shiftwork schedule and thus sleep occurred during the biological day when melatonin levels were low and wakefulness occurred during the biological night when melatonin levels were high (Fig. 2). Daytime sleep opportunities resulted in shorter latencies to sleep onset (SOL), persistent sleep (LPS) and REM sleep (REML), lower total sleep time, sleep efficiency, and percentage and minutes of stage 2 sleep, and higher minutes of wakefulness after sleep onset (WASO), as well as an increase in the average duration of awakenings (Table S1).

Relative Clock Hour
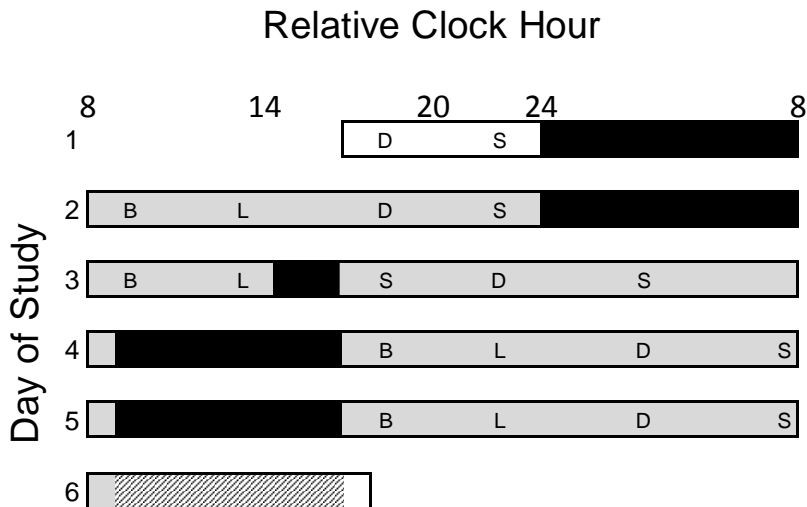
Fig. 1. Study protocol. Time of day is plotted as relative clock hour with scheduled waketime arbitrarily assigned a value of 0800h and all other times referenced to this value (e.g., breakfast 1.5h after scheduled awakening would be reported as occurring at a relative time of day of 0930h; actual clock hour is determined by participant's habitual wakefulness-sleep schedule assessed during ambulatory baseline). This procedure permitted subjects to sleep at their habitual circadian phase at baseline. Open bars represent room light (<40lux). Black bars represent scheduled PSG recorded sleep. Hatched bars represent non-PSG recorded sleep. Shaded areas indicate scheduled wakefulness in dim light (<1 lux). Day 1 consisted of a sleep disorders screen. Day 2 (baseline) consisted of 16h of daytime wakefulness during the day with an 8h nighttime sleep opportunity at habitual time. Day 3 (transition and nightshift 1) consisted of 7h of wakefulness followed by a 2h nap and 15h of wakefulness to simulate the transition to the nightshift. Days 4 (nightshift 2) and 5 (nightshift 3) consisted of an 8h daytime sleep opportunity and 16h of afternoon and nighttime wakefulness. Meal content was the same each day. B=breakfast; L=lunch; D=dinner; S=snack.
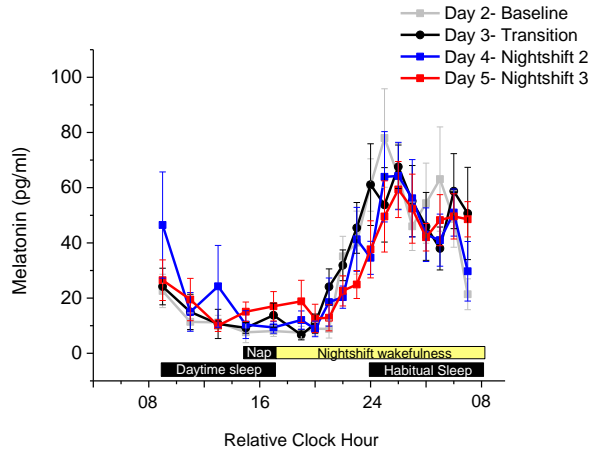
Fig. 2. Hourly melatonin levels. The gray line represents day 2 (baseline), the black line day 3 (transition to first nightshift), the blue line day 4 (second night shift), and the red line day 5 (third night shift). Time of day is plotted as relative clock hour with scheduled waketime arbitrarily assigned a value of 0800h. The black bars represent scheduled sleep and the yellow bar represents scheduled nighttime wakefulness during shiftwork. Error bars are SEM.

Hourly, Total Daily, Wakefulness and Sleeping EE, Activity, and Substrate Utilization and Balance. Regardless of whether sleep occurred at night or during the daytime, average hourly EE was lower during sleep than during wakefulness (Fig. 3A, Fig. S1). EE during the 2h daytime nap on the transition day (day 3) was reduced to similar levels as seen in the first 2h of the baseline nighttime sleep episode. Total daily EE was ~347 kjoules higher on the transition day to the first night shift, which included a 2h nap and extended wakefulness, and was ~223 and ~269 kjoules lower on the second and third night shift days (days 4 and 5), respectively, than baseline day 2 ($p < 0.025$; Fig. 3B). Waking EE was similar between days ($p > 0.22$; Fig. 3C), whereas subjects expended ~12% less energy during daytime sleep opportunities on the first and second nightshift days as compared to baseline ($p < 0.0001$; Fig. 3D). Further, average wrist activity levels on the transition day to the first night shift were ~32 and 17% higher than on baseline and the second night shift (Fig. S2, $p < 0.05$), respectively, and were ~14% higher (non-significant trend) than on the third nightshift (Fig. S2. $p = 0.06$).

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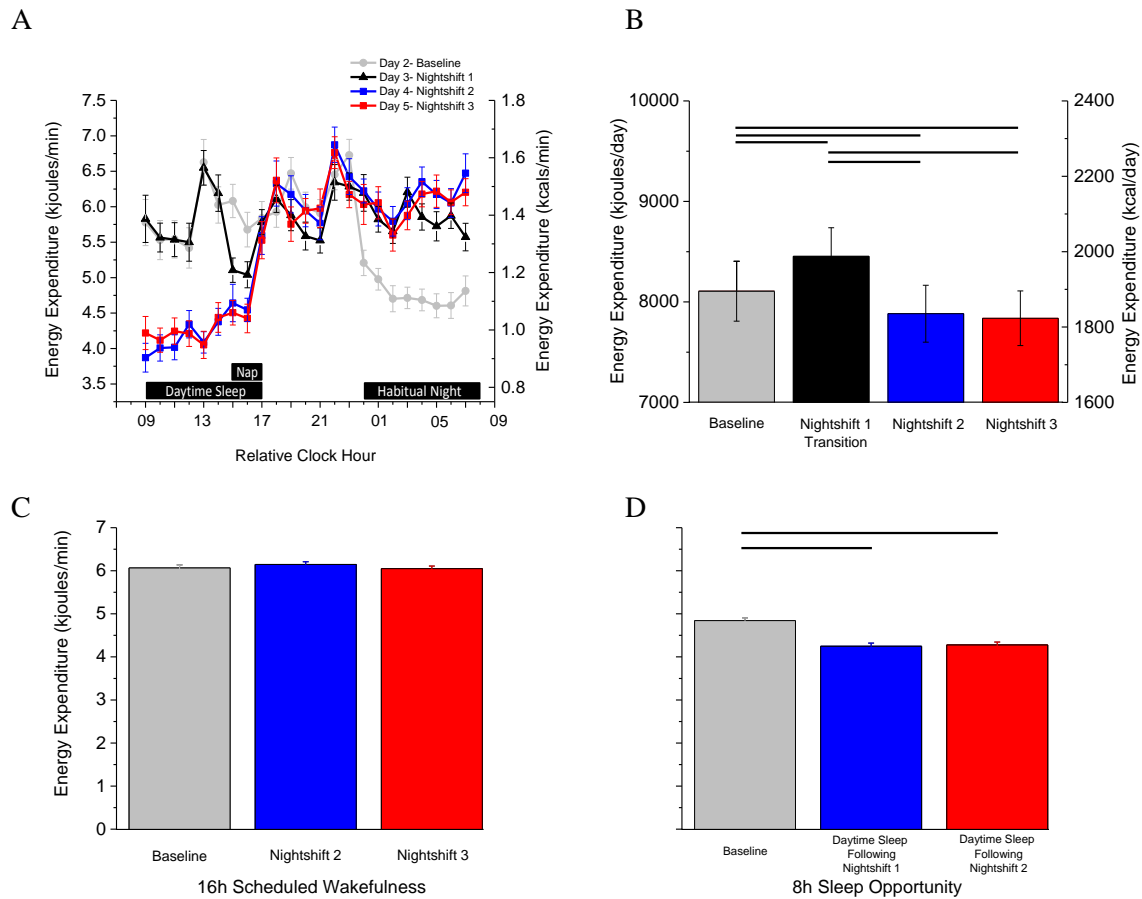
Fig. 3. Hourly, total daily, wakefulness and sleeping average energy expenditure across the simulated shiftwork protocol (n=14). The gray line represents day 2 (baseline), the black line day 3 (transition to first nightshift), the blue line day 4 (second night shift), and the red line day 5 (third night shift). Black boxes along the x-axis indicate scheduled sleep opportunities. Solid lines represent significant differences for points at the end of each line (planned comparisons mixed model ANOVAs with modified bonferroni corrections for multiple comparisons ($p < 0.025$)). Note that the nap only occurs on the transition to the first nightshift. Error bars are SEM.

Table 1 shows that total daily fat utilization was ~9% higher on the transition day to the first nightshift and was ~19% higher on the second nightshift day as compared to baseline, resulting in negative fat balance on the first two shiftwork days. Furthermore, total daily carbohydrate and protein utilization were ~21 and ~13% lower, respectively, on the second nightshift as compared to baseline resulting in greater positive carbohydrate and protein balance. Substrate utilization was similar to baseline on the third nightshift day (Table 1).

**Table 1. Total daily substrate utilization and balance for baseline and nightshift days.**

Measure	Day 2-Baseline (n=13)	Day 3-Nightshift 1/ Transition Day (n=13)	Day 4-Nightshift 2 (n=13)	Day 5-Nightshift 3 (n=12)
Carbohydrate Utilization (g/day)	207.6 (8.7)	211.0 (10.6)	164.9 (7.1)$^{\#\dagger}$	200.6 (10.0)$^{\$}$
Carbohydrate Balance (g/day)	29.7 (11.8)	25.3 (10.8)	70.6 (10.3)$^{\#\dagger}$	29.7 (12.1)$^{\$}$
Fat Utilization (g/day)	84.8 (7.3)	92.8 (6.9)*	101.2 (7.1)$^{\#\dagger}$	81.4 (7.2)$^{\ddagger\$}$
Fat Balance (g/day)	-22.1 (6.1)	-29.9 (5.9)*	-39.3 (5.8)$^{\#\dagger}$	-20.7 (6.3)$^{\ddagger\$}$
Protein Utilization (g/day)	60.8 (2.5)	59.9 (4.5)	53.0 (3.3)$^{\#\dagger}$	59.2 (3.9)$^{\$}$
Protein Balance (g/day)	9.6 (2.5)	10.5 (5.0)	16.5 (3.6)$^{\#\dagger}$	9.2 (2.1)$^{\$}$

* indicates differences between baseline and transition to the first nightshift, # indicates differences between baseline and nightshift 2, † indicates differences between transition to the first nightshift and nightshift 2, ‡ indicates differences between transition to the first nightshift and nightshift 3, $ indicates differences between nightshift 2 and nightshift 3. Values in parenthesis are SEM.

Sleep and Wakefulness Stages and EE. We first examined EE for each 8h sleep opportunity separately and found that EE did not significantly differ between stages of consolidated sleep ($p > 0.14$) (Fig 4; Table S2). During the baseline sleep opportunity, EE was higher during wakefulness prior to and wakefulness after sleep onset (WPSO and WASO, respectively) than during stages 2 and REM sleep. Further, EE was higher during WPSO than during stages 1, slow wave sleep (SWS), and WASO (Table S2, $p < 0.05$). During the two daytime sleep opportunities after night shiftwork, however, EE during WPSO was lower than during the consolidated sleep stages while EE during WASO was higher than during most sleep stages and WPSO, with the exception of stage 1 on the second night shift day (Table S2, $p < 0.05$).

We next examined EE differences for consolidated sleep stages between nighttime and daytime sleep opportunities and found that EE was lower during WPSO, stage 2, and SWS during daytime sleep opportunities than during baseline (Fig. 4, $p < 0.05$). Further, EE was lower during WASO and REM sleep during the third night shift day compared to the baseline. When examining differences in EE for all scored sleep epochs between baseline and daytime sleep opportunities, subjects expended ~14% and ~11% less energy during sleep on shiftwork days 4 and 5 as compared to baseline (Fig. S3, $p < 0.001$).
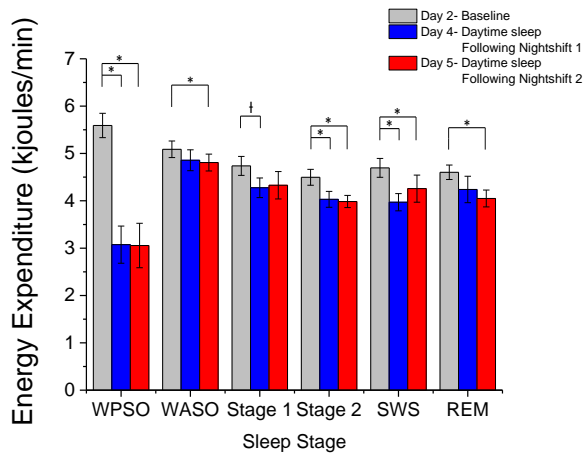
Fig. 4. Average energy expenditure per sleep stage. Planned comparisons calculated by mixed model ANOVAS for sleep stage. Solid lines and * represent significant (p<0.05) differences for points and †representnon-significant trends (p=0.07) between stages at the end of each line. Error bars are SEM.

TEF in Response to Dinner. Average TEF was decreased on the transition day and a non-significant trend for decreased TEF on the second nightshift as compared to the baseline day 2 in response to dinner (Table 2, p<0.025, p= 0.04). Further, TEF returned to baseline on the third nightshift (Table 2).

Table 2. Thermic effect of food in response to dinner.

Measure	Day 2- Baseline (n=15)	Day 3- Nightshift 1/ Transition Day (n=15)	Day 4- Nightshift 2 (n=11)	Day 5- Nightshift 3 (n=12)
Percent of energy content of meal (%)	5.0 (1.0)	0.9 (1.3)[*]	2.8 (0.7)[†]	5.0 (0.9) [‡]

* indicates differences between baseline and transition to the first nightshift (p<0.025), ‡ indicates differences between transition to the first nightshift and nightshift 3 (p<0.025), †indicates non-significant trend between baseline and nightshift 2 (p=0.045). Values in parenthesis are SEM.

**Satiety and Hunger Hormones.** Regardless of whether sleep occurred at night or during the daytime, average 2h hourly PYY levels were higher during wakefulness than during sleep (Fig 5A). Average 24h PYY levels were decreased by ~3% on the transition day and by ~11% on the second nightshift day as compared to baseline day 2 (Fig. 5B) and average 24h PYY levels returned to baseline on the third shiftwork day. Examination of average PYY levels during scheduled wakefulness showed that PYY was decreased on the second nightshift as compared to baseline and returned to baseline levels on the third shiftwork day. Further, PYY levels during scheduled sleep opportunities showed decreased PYY on the sleep opportunity following nightshift 1 as compared to scheduled sleep on baseline and increased PYY levels on the sleep opportunity following nightshift 2 as compared to both scheduled sleep on the baseline and following nightshift 1 (Fig. S4A,B). Average 2h hourly leptin levels were generally lower during wakefulness than during sleep (Fig 5C). On the first nightshift day of extended wakefulness, leptin levels were relatively stable. Average 24h leptin levels decreased by ~16 and 29% on the first two days of shiftwork as compared to baseline ($p<0.025$), but were not statistically different from baseline on the third nightshift day (Fig. 5D). Further, leptin levels were decreased during scheduled wakefulness on the first nightshift day ($p<0.025$), with a non-significant trend ($p=0.03$) on the second nightshift as compared to baseline (Fig. S4C). Similar leptin levels were observed during scheduled sleep regardless of day (Fig. S4D). Average 2h hourly ghrelin levels fluctuated across the day (Fig. 5E) and average 24h levels did not show a difference across study days, regardless of wakefulness or sleep (Fig. 5F, Fig. S4E, F, $p>0.25$).
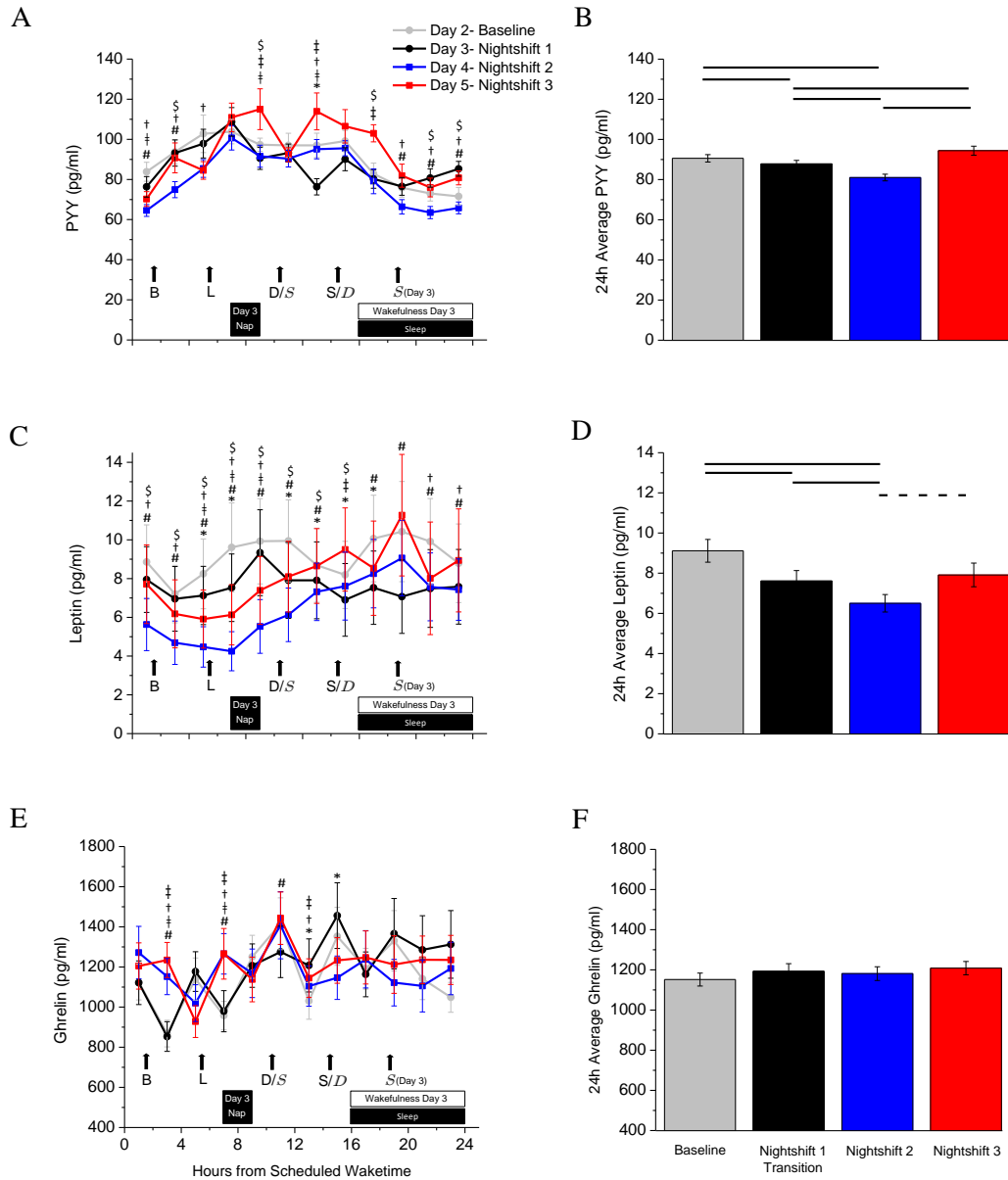
Fig. 5. Hourly (Left, n=14, two-tailed) and average (Right, n=14, one-tailed) satiety and hunger hormones. Planned comparisons calculated by dependent t-test (left) and mixed model ANOVAS for study day with modified bonferroni corrections for multiple comparisons (p<0.025) (right). * indicates differences between baseline and transition to nightshift 1, # indicates differences between baseline and nightshift 2, ‡ indicates differences between baseline and nightshift 3, † indicates differences between transition to first nightshift 1 and nightshift 2, ‡ indicates differences between transition to nightshift 1 and nightshift 3, $ indicates differences between nightshift 2 and nightshift 3. Solid lines represent significant (p<0.025) differences and dashed lines indicate non-significant trends (p=0.35) for points at the end of each line. Black arrows indicate the timing of meals. B=breakfast; L=lunch; D=dinner; S=snack; italicized D and S indicate different meal given at that time on transition to the first nighshift. Black bars represent sleep opportunities. Note that the nap only occurs on the transition to the first nightshift and the open bar represents sleep deprivation. Error bars are SEM.

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Hunger Scales. Subjective hunger ratings decreased across days of study and were ~8 and 22%

lower on the second and third nightshift days, respectively, as compared to baseline and were

~18 and 14% lower on the second and third nightshift days, respectively, as compared to the

first nightshift (Fig. 6, $p < 0.025$).
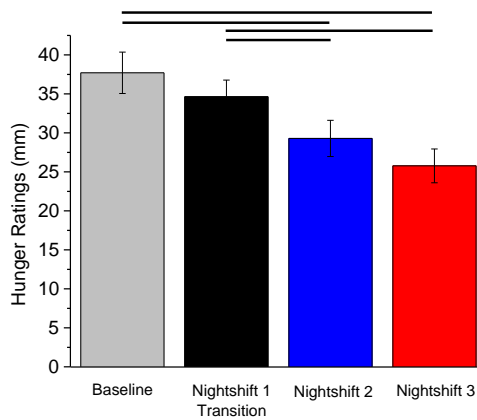
Fig. 6. Average (n=15, one tailed) 24h hunger ratings. Planned comparisons calculated by mixed model ANOVAS for study day with modified bonferroni corrections for multiple comparisons ($p < 0.025$). Solid lines represent significant differences at the end of each line ($p < 0.025$). Error bars are SEM.

DISCUSSION

Findings from both human and animal models indicate that food intake during the time normally reserved for sleep increases the risk of weight gain and metabolic dysregulation. We show that total daily EE was significantly increased on the first nightshift with extended wakefulness and decreased on the second and third nightshift days as compared to baseline. Additionally, we find that the decreases in total daily EE during nightshift work are driven predominantly by decreases in sleeping EE versus waking EE, despite disrupted daytime sleep. We also show that the TEF in response to dinner decreased on the first nightshift and a non-significant trend for decreased TEF on the second nightshift as compared to the baseline day of daytime wakefulness. Total daily fat utilization was increased on the first and second nightshifts and total daily carbohydrate and protein utilization were decreased on the second nightshift. Lastly, subject's exhibited a decrease in hunger ratings with no changes in the hunger stimulating hormone ghrelin and despite decreases in the satiety hormones leptin and PYY.

Total Daily EE. Using a common 3-day nightshift work schedule model we found that acute circadian misalignment increased total daily EE on the transition day to the first nightshift and decreased total daily EE on the second and third nightshifts. The finding of increased EE during the transition day to the first nightshift was expected due to the increased time awake and prior findings from our laboratory that wakefulness is more energetically costly than sleep (22, 23). Our findings of decreased total daily EE during subsequent nightshifts is novel and may represent a contributing mechanism for weight gain observed in shift workers. Specifically, if total daily caloric intake was not reduced in shiftworkers, the ~223 to ~269 kjoules/day reduction

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in total daily EE we observed would lead to positive energy balance and weight gain over time

since as little as ~209kjoules/day excess calorie storage can explain the obesity epidemic (24).

Indeed, shiftworkers report eating similar meal sizes as daytime workers (9, 25-27), further

supporting this mechanism as a contributor for weight gain.

The thermic effect of food (TEF) accounts for ~10% of the total daily EE and reflects the

energy needed for the absorption, digestion, and storage of consumed meals (28). We found that

the TEF in response to a late dinner consumed at relative clock hour of 2230h on the first night

shift was lower as compared to a dinner consumed at a relative clock hour of 1830h on the

baseline day. We also found a non-significant trend for decreased EE in response to dinner at

relative clock hour 0330h on the second night shift compared to baseline. EE returned to levels

similar to baseline on the third night shift suggesting some adaptation in the TEF during the

acute challenges posed by such recurrent work schedules. A lower TEF in response to meals

consumed at night may contribute to reduced total daily EE during shift work. Our lower TEF

findings are in agreement with findings from a study by Romon and colleagues who reported that

a meal consumed at 0100h showed a lower TEF as compared to the identical meal consumed at

0900h or 1700 h (29). The decreased TEF we observed in response to a late dinner prior to the

first nightshift may represent a contributing mechanism underlying the reported risk of weight

gain associated with food intake in the evening hours. For example, food consumed past 2000 h

has been found to predict BMI when controlling for sleep duration and timing (30) and Night

Eating Syndrome, a disorder in which individuals consume ≥25% of their caloric need after their

evening meal (31), is associated with higher BMI (32). With regards to shift work, it is

unknown if shifting the timing of larger meals to the early and later parts of the overnight shifts

(i.e., during the biological day), may help to alleviate weight gains in shiftworkers.

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Substrate Utilization and Balance. Changes in substrate utilization may also contribute to metabolic dysfunction in shift workers. An increase in fat utilization and negative fat balance on the first and second night shift days was not expected, nor was the decrease in carbohydrate and protein utilization and positive carbohydrate and protein balance, relative to baseline. These findings may represent an acute physiological response to the increased EE during sleep deprivation of the first nightshift day, as the total daily food intake provided was the amount needed to maintain energy balance at baseline, and since fat utilization provides more energy per gram(33, 34). Acute switches in substrate utilization between fat and carbohydrate utilization, or metabolic flexibility, are necessary to respond to varying macronutrients consumed across the day (35). Decreased ability to alter between substrates for utilization, a metabolic inflexibility, has been observed in obese individuals and results in a blunted fat utilization (36). As the present study only tested healthy lean individuals, it is unknown how an overweight or obese individual, as common in shiftwork (37, 38), would physiologically respond to sleep deprivation and subsequent nighshift work with the increased demand for fat utilization. Additionally, if such acute changes in substrate utilization occur on a weekly basis as shift workers move back and forth between a daytime schedule on days off and a nighttime schedule during work, it is unknown what physiological adaptation may occur or how this will impact metabolic flexibility.

Insufficient sleep schedules are reported to increase carbohydrate consumption in evening after dinner (23, 39) leading to weight gain. We provided subjects a controlled energy balance diet based on their daily caloric needs at baseline and thus additional research is needed to examine changes in the amount and types of food consumed under simulated nightshift work and *ad libitum* energy intake.

Sleep Architecture and EE. Consistent with prior findings, we observed that sleeping during the biological day after simulated nightshift work, when melatonin levels were low, disturbed sleep (40-44). Shorter latencies to sleep were expected due to increased homeostatic sleep drive and the circadian phase of sleep propensity (45-48). Reductions in total sleep time and stage 2 sleep and increases in the duration of arousals were also expected due to the circadian promotion of arousal during the latter potion of the daytime sleep episode (See Chapter 3)(47, 48). The ~30min shorter REML on the days 4 and 5 during the daytime sleep opportunities is consistent with the circadian rhythm in REM sleep propensity, as these sleep opportunities were schedule near the circadian peak for REM (47).

Contrary to our hypothesis, we found that EE decreased during daytime sleep opportunities indicating that sleeping at an inappropriate circadian time lowers sleeping energy expenditure beyond that observed when sleep occurs at night. However, our findings of higher EE during awakenings from sleep compared to consolidated sleep and similar EE among consolidated sleep stages within the sleep episode are consistent with prior findings(22, 49). The finding of a lower EE during wakefulness prior to sleep onset for daytime sleep episodes than the EE for consolidated sleep stages was unexpected. This finding is contrary to the EE observed at baseline during the transition from wakefulness to sleep and is inconsistent with findings from prior research with sleep episodes at night (22). Possible contributing mechanisms for decreased EE at the beginning of the daytime sleep episode include sleep induced decreases in levels of the metabolic hormones cortisol, norepinephrine, and epinephrine which typically increase EE (50-52). During sleep, particularly SWS, cortisol pulsatile secretion is reduced resulting in lower cortisol levels (53, 54). Thus, sleep in the morning after the nightshift could potentially decrease

cortisol levels and lead to an associated decrease in EE. Circulating norepinephrine and epinephrine levels are reduced during sleep (55) and to the greatest extent during REM sleep (56). Our finding of shorter latencies to REM sleep after simulated nightshifts may be associated with reduced catecholamine levels, thereby potentially decreasing EE during the beginning of the sleep episode. These possible mechanisms, however, cannot explain why EE is lower prior to daytime sleep. Further studies are needed to elucidate physiological mechanisms by which energy is decreased when sleep occurs at inappropriate circadian times using animal models.

Satiety and Appetitive Hormones. Circadian misalignment and insufficient sleep have been shown to alter appetitive hormones and increase appetite when subjects are given meals designed to meet energy balance at baseline(4, 14, 57, 58). Our findings of decreased total daily leptin and PYY levels, with no changes in total daily ghrelin, would be hypothesized to increase hunger ratings, yet we observed decreased hunger ratings during the nightshift schedule. As shift workers report no difference in total daily food intake compared to day workers (9, 25-27), other mechanisms must therefore contribute to the maintenance of food intake. Increases in other peripheral satiety hormones that were not measured in the current study such as glucagon-like-peptide (GLP-1), desacyl ghrelin, or cholecystokinin (CCK), or central mechanisms such as decreases in the hunger promoting orexin/hypocretin during circadian misalignment may contribute to the increased satiety observed.

Summary. Knowledge about potential mechanisms for increased risk in weight gain and obesity during night shiftwork are important for developing evidence-based treatment strategies. Our

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findings provide novel insight into how circadian misalignment during nightshift work disturbs metabolic physiology and may contribute to adverse metabolic health outcomes like obesity. We uniquely show that in a controlled laboratory environment, wakefulness and food consumption at night and sleeping during the day decreases total daily EE. Further, these decreases are occurring predominately during the daytime sleeping episodes, despite increased duration of arousals. Research extending these findings to examine the effects of different diet compositions, physical activity levels, overweight or obese health status, and age related changes are needed to fully elucidate other potential contributing mechanisms to metabolic dysregulation in shift workers. Additionally, treating circadian misalignment and targeting treatment therapies towards increasing EE during the nightshift may promote metabolic health of shift workers.

METHODS

Subjects. Fifteen healthy subjects (n=9 females) aged (26.13±4.5 y; mean±SD), BMI (22.67±1.7 kg/m^2), and percent body fat (28.4±7.7%) as determined by dual energy X-ray absorptiometry (DEXA, Prodigy Advance GE/Lunar), participated. Study procedures were approved by the scientific and advisory review committee of the Colorado Clinical and Translational Sciences Institute, by the Colorado Multiple Institutional Review Board (IRB), and by the University of Colorado Boulder IRB. Subjects gave written informed consent and then underwent health screening at the Sleep and Chronobiology Laboratory and at the CTRC at the University Colorado Boulder. Exclusion criteria consisted of diagnoses with any known medical, psychiatric or sleep disorder, current smoker, pregnancy, or a habitual sleep duration of <7h or > 9.25h. Subjects were deemed healthy based on clinical history physical exam,

psychological tests and psychological interview with a trained clinician, blood chemistries (complete blood cell count and comprehensive metabolic panel), clinical electrocardiogram, and medication free status. No subjects reported regular night work in the preceding year or crossing more than one time zone in the previous three weeks. Subjects were minimally physically active prior to study to minimize the detraining effects of the posture controlled study.

Study protocol

For one week prior to admission to the University of Colorado Hospital (UCH) CTRC, subjects maintained a self-selected sleep schedule of ~8h per night and caffeine, alcohol, nicotine, and over-the-counter medication use were proscribed. The sleep and wakefulness schedule was verified via continuous wrist actigraphy with light exposure monitoring (Actiwatch-L, Mini Mitter Respironics, Bend, OR), sleep-wakefulness logs, and call-in bed and wake times to a time stamped voice-mailbox recorder. Drug use was determined by self-report and verified by urine toxicology at screening and by urine toxicology and a breath alcohol tester (Lifeloc Technologies Model FC10, Wheat Ridge, CO) upon hospital admission. Three days prior to hospital admission, exercise was proscribed and subjects were provided a 3-day outpatient isocaloric diet that was designed to meet their individual daily caloric needs as determined from resting metabolic rate with a 1.5 activity factor. Subjects were instructed to eat the diet in its entirety and nothing else but water to ensure energy balance at the start of laboratory protocol. Diets were prepared by the UCH CTRC Nutrition Core and contained macronutrient contents recommended by the American Heart Association (30% fat, 55% carbohydrate, and 15% protein) and no caffeine.

In-Laboratory Protocol

Subjects lived in the UCH CTRC for ~6-d to simulate a daytime work schedule followed by the transition to and first three nights of a nighttime work schedule. Subjects were admitted to the laboratory ~7h prior to their habitual bedtime as determined from the week of 8h self-selected sleep schedules. Polysomnography (PSG) was used to record an 8h screening nighttime sleep episode at the subjects' habitual bedtime and to verify that they were free from sleep disorders. Following the sleep disorders screening night, subjects were awakened at habitual waketime and maintained a constant posture protocol; seated semi-recumbent posture in a hospital bed with the head raised to ~35 degrees, room temperature maintained in the thermoneutral range (22-24° Celsius), dim lighting (<1 lux in the angle of gaze, <5 lux maximum) during scheduled wakefulness and 0 lux during scheduled sleep. Subjects were allowed three brief scheduled breaks per day to stand up and retrieve meals and use the bathroom (a toilet <3m from the bed). Outside of scheduled breaks, subjects were given a urinal or bedpan to use if needed. Wakefulness and subject compliance during the constant posture protocol was verified via continuous monitoring by research staff and electroencephalography (EEG) recordings. Day 2 served as a baseline dayshift with 16h of daytime wakefulness and an 8h nighttime sleep opportunity. Day 3 served as the transition to working the first nightshift. Subjects were awakened at habitual waketime and were scheduled to a 2h afternoon sleep opportunity prior to the first nightshift. After the first nightshift subjects were provided an 8h daytime sleep opportunity that began one hour after their habitual baseline waketime. This was followed by two days of nightshift work (Fig 1). After the last nightshift, subjects were provided

an 8h recovery sleep opportunity on day 6 that was not PSG recorded and then were discharged from the hospital.

Subjects received scheduled isocaloric meals (percent of daily caloric intake, 30% breakfast, 30% lunch, 30% dinner, 10% snack) at approximately 1.5h, 5.5h, 10.5h and 14.5h post awakening on baseline day 2 (relative clock hour 0930, 1330, 1830, and 2230h for a subject with an 0800h baseline waketime) and on days 4, and 5 (relative clock hour 1730, 2130, 0330, and 0730h for a subject with an 0800h baseline waketime).   Meals were the same across days (e.g. food served for breakfast was the same each day) and subjects were instructed to consume all food provided. As Day 3 of the protocol simulated the transition to the nightshift with a 2h sleep opportunity in the middle of the day, the timing and percent of daily caloric intake (30% breakfast, 25% lunch, 25% dinner, 10% snack, 10% snack) were spread across the day; breakfast was given at 1.5h , lunch at 5.5h, and dinner at 15.5h post awakening with two snacks given at 10.5h and 20.5h (relative clock hour 0930, 1330, 1830, 2230, and 2330h for a subject with an 0800h baseline waketime) after awakening. Dinner was designed as a cold meal so that it could be placed in a refrigerator next to the bed to control for changes in posture to permit the TEF analysis.

Measures

EE and substrate utilization were determined via whole room indirect calorimetry during days 2-5 of the study (23, 59). Subject's carbon dioxide (CO_2) production and oxygen (O_2) consumption were assessed to calculate EE and respiratory quotient (RQ) by measuring gas concentration differences in entering and exciting of the chamber via a fuel-cell–based dual

channel O_2 analyzer (FC-2 Oxzilla; Sable Systems International, Las Vegas NV) and two

infraredCO2 analyzers (CA-10 CO_2 analyzers; Sable Systems International, Las Vegas NV) (59).

Monthly propane combustion tests validated the precision and accuracy of the gas concentration

measurements with average recoveries of~99%. Non-protein carbohydrate and fat utilization

were determined from O_2 consumption and RQ (60) and protein utilization was calculated using

24h urine total nitrogen collected throughout the duration of the study (61). UCH CTRC

dieticians determined the caloric and macronutrient content of the study meals using ProNutra

software (62).

Leptin, ghrelin, and PYY levels were calculated from blood samples drawn every two

hours throughout the study. Melatonin was assessed every two hours during the daytime and

hourly during the night starting at 13h after habitual waketime (relative clock hour 1900 for 0800

waketime). Blood was drawn using a 12-foot extension tubing through a porthole in the whole

room calorimeter that was connected to an indwelling venous catheter and kept patent with a

heparinized saline drip (23). The extension tubing allowed for blood sampling without entering

the chamber or disrupting scheduled sleep opportunities. Hunger ratings were determined via

visual analog scales (VAS) collected every 2h starting at 2h post awakening and ending 2h prior

to sleep.

Sleep and wakefulness recordings were obtained using Siesta digital sleep recorders

(Compumedics USA Ltd, Charlotte, NC) from C3-A2, C4-A1, O1-A2, O2-A3, F3-A2, F4-A1,

right and left electrooculogram (EOG), chin electromyogram (EMG), and electrocardiogram

(ECG). Night 1 consisted of a sleep disorders screen for sleep apnea and periodic limb

movements based on standardized air flow, respiratory effort and leg EMG measurements. Sleep

was manually scored in 30s epochs according to standard guidelines from brain region C3-A2

(63) or C4-A1 if C3-A2 contained artifact. SOL was defined as the time from lights out to the onset of 3 continuous epochs of PSG defined sleep; LPS was defined as the time from lights out to the onset of 10 continuous minutes of PSG defined sleep; SWSL was defined as the time from LPS to SWS; and REML was defined as time from LPS to REM sleep.

Data Analysis

EE and substrate utilization were collected every minute and averaged hourly, during wakefulness, during scheduled sleep opportunities, and for the 24h day. Total daily carbohydrate balance was calculated accounting for fiber intake. EE was also calculated for consolidated sleep and wakefulness stages during scheduled sleep opportunities and for all scored sleep epochs. Specifically, sleep and wakefulness stages [WPSO, WASO, stage 1 sleep, stage 2 sleep, SWS, and REM] were matched with EE values that were offset by 2 minutes to account for the lag time response of the whole room calorimeter relative to the timing of sleep recording (22, 64). As EE was collected in minute increments and sleep was scored in 30-second epochs, sleep was binned as done in (22); EE corresponding to consolidated stage 2 and SWS epochs were binned and averaged for ≥15 min of continuous sleep for that stage, with the exception of 4 subjects who did not have 15 min of continuous SWS and therefore the maximum time for each subject was used (minimum bin size was 4 min for 1 subject); EE bins were ≥9.5 min for REM, ≥1 min for stage 1, and ≥ 30s for WPSO and WASO. Blood data were aligned and plotted from hours awake to compare levels across wakefulness. TEF was measured for 1h prior to dinner (pre-meal RMR baseline, subjects seated for ~4h prior to baseline measurement) and 3.5h post dinner consumption on days 2-5 at relative clock hour 1830, 2230, 0330 and 0330h, respectively,

for a person with a 0800h baseline waketime. TEF data were binned into 30 min bins and analyzed as percentage of the consumed dinner and as a deviation from the 1h baseline. Feeding and satiety hormones were analyzed for both 24h averages and 2h hourly levels. Total daily EE and hormone data were not available for two subjects and were therefore excluded from those analyses. Protein utilization levels were not available for two subjects throughout the protocol and for one subject on the nightshift 3 day, and thus they were excluded from substrate utilization analysis. Four subjects on nightshift 2 and 3 subjects on nightshift 3 did not have documented completion times of the dinner on those nights and were therefore excluded from the TEF analysis on those days.

Data were analyzed with mixed model ANOVAs that included study day, relative clock time, and/or sleep stage as a fixed factors and subject as a random factor using STATISTICA, version 10.0 (StatSoft Inc, Tulsa, OK). One-tailed a priori directional planned comparisons were used to examine total daily EE, average wakefulness and sleep EE, TEF, feeding and satiety hormones, and hunger ratings, and modified bonferroni correction factors were used to correct for multiple planned comparisons to reduce type 1 error (65). Individual time-points were compared using dependent t-tests.

Acknowledgements

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CHAPTER 2
Supplementary Materials

Impact of Circadian Misalignment on Energy Metabolism and Sleep During Simulated Night Shift Work

Supplementary Table S1
Supplementary Table S2
Supplementary Figure S1
Supplementary Figure S2
Supplementary Figure S3

Table S1- **Sleep architecture for nighttime baseline and daytime shiftwork sleep opportunities**. Findings for changes in both daytime sleep episodes are described in the main text. Daytime sleep on day 4 reduced both minutes and percentage of REM sleep by ~20%, and had a non-significant trend for increases in both percent and minutes of.

| Measure	Baseline Nighttime Sleep Opportunity (n=15)	Daytime Sleep Following Nightshift 2 (n=14)	Daytime Sleep Following Nightshift 3 (n=14)
Percent of Recording Time			
stage 1	4.1 (0.5)	4.0 (0.8)	4.0 (0.5)
stage 2	55.4 (1.8)	46.6 (2.1)*	47.1 (2.5)*
SWS	12.8 (1.5)	14.6 (1.8)†	14.1 (1.7)
REM	18.3 (1.2)	14.6 (1.2)*	16.1 (1.0)
SE	90.7 (1.9)	79.7 (2.4)*	81.3 (2.2)*
Minutes of Recording Time			
stage 1	20.1 (2.5)	19.0 (2.5)	19.2 (2.7)
stage 2	265.7 (8.7)	223.7 (10.1)*	226.0 (12.1)*
SWS	61.4 (7.1)	70.1 (8.7)†	67.4 (8.0)
REM	88.1 (5.8)	69.9 (5.9)*	77.1 (4.8)
WASO	33.6 (8.9)	92.1 (11.3)*	85.2 (10.1)*
TST	435.2 (9.1)	382.7 (11.4)*	389.6 (10.8)*
SOL	9.5 (4.6)	5.4 (3.2)*	4.7 (4.1)*
LPS	13.5 (1.7)	5.8 (0.9)*	4.9 (1.3)*
SWSL	17.9 (2.4)	19.7 (3.3)	16.86 (1.9)
REML	83.0 (7.4)	51.4 (4.4)*	54.6 (1.4)*
Duration of Awakenings	1.4 (0.3)	4.9 (1.0)*	4.9 (1.0)*
Number of Awakenings	22.8 (2.6)	23.6 (3.4)	23.4 (3.0)

LPS = latency to persistent sleep; REM = rapid eye movement; REML = latency to REM sleep; SE = sleep efficiency; SOL= sleep onset latency; SWS = slow wave sleep; SWSL = latency to SWS; TST = total sleep time; WASO = wakefulness aftersleep onset. *denotes $p = <0.05$, † denotes $p = 0.05$ between baseline night sleep opportunity and daytime sleep opportunities, values in parenthesis are standard error of the mean.

Table S2- ANOVA F-values for energy expenditure comparisons between sleep stages for individual sleep opportunities.

| Measure	stage 1	stage 2	SWS	REM	WPSO	WASO
Baseline Nighttime Sleep Opportunity (n=15)						
stage 1						
stage 2	1.03					
SWS	0.03	0.84				
REM	0.57	0.49	0.21			
WPSO	11.50*	14.84*	23.46*	13.7*		
WASO	3.32†	13.22*	4.57†	6.74*	13.70*	
Daytime Sleep Following Nightshift 2 (n=14)						
stage 1						
stage 2	2.37					
SWS	4.48	0.43				
REM	0.04	1.40	3.96			
WPSO	15.66*	5.82*	5.86*	8.63*		
WASO	16.85*	27.59*	28.77*	12.09*	29.05*	
Daytime Sleep Following Nightshift 3 (n=14)						
stage 1						
stage 2	1.78					
SWS	0.06	1.05				
REM	1.97	0.26	0.97			
WPSO	10.77*	5.09*	6.75*	6.93*		
WASO	4.20	28.29*	12.03*	38.58*	20.30*	

*denotes $p = <0.05$ between stages, † denotes $p >0.051$ between sleep stages
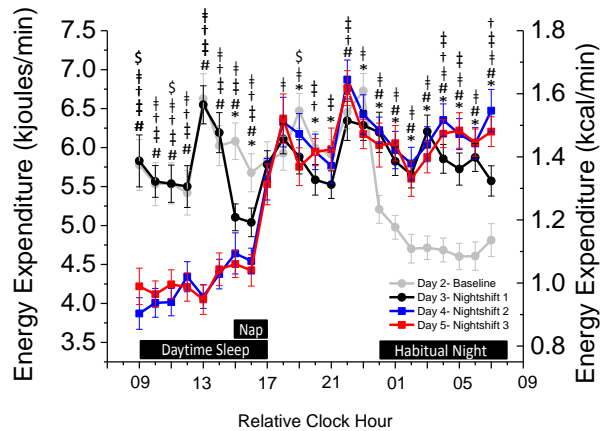
Fig. S1. Total daily energy expenditure with hourly comparisons. Planned comparisons calculated with dependent t-test (p<0.05). * indicates differences between baseline and transition to first nightshift, # indicates differences between baseline and nightshift 2, ǂ indicates differences between baseline and nightshift 3, † indicates differences between transition to nightshift 1 and nightshift 2, ‡ indicates differences between transition to nightshift 1 and nightshift 3, $ indicates differences between nightshift 2 and nightshift 3. Energy expenditure was lower during sleep than wakefulness. Error bars are SEM.
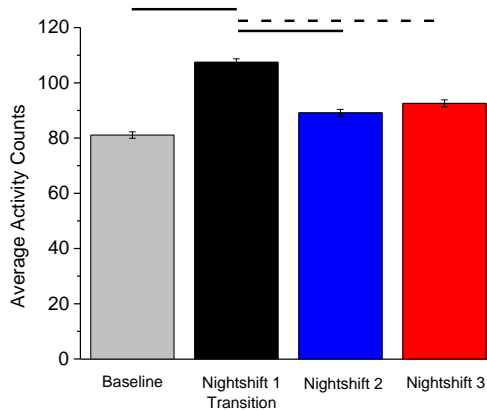
Fig. S2. Average activity counts from actigraphy across the protocol. Solid lines represent significant (p<0.05) differences for points between stages at the end of each line and dashed lines indicate non-significant trends (p=0.06) for points at the end of each line. Activity was higher of the transition to the first night shift than baseline, nightshift 2, and a non-significant trend for increased activity as compared to nightshift 3. Error bars are SEM.
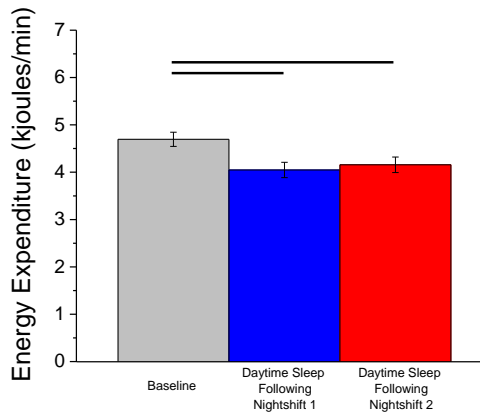
Fig. S3. Average energy expenditure for all scored sleep during the sleep opportunity. Planned comparisons calculated by mixed model ANOVAS for study day. Solid lines represent significant differences for points between stages at the end of each line(p<0.001). Energy expenditure was significantly lower during daytime sleep opportunities than at habitual sleep time. Error bars are SEM
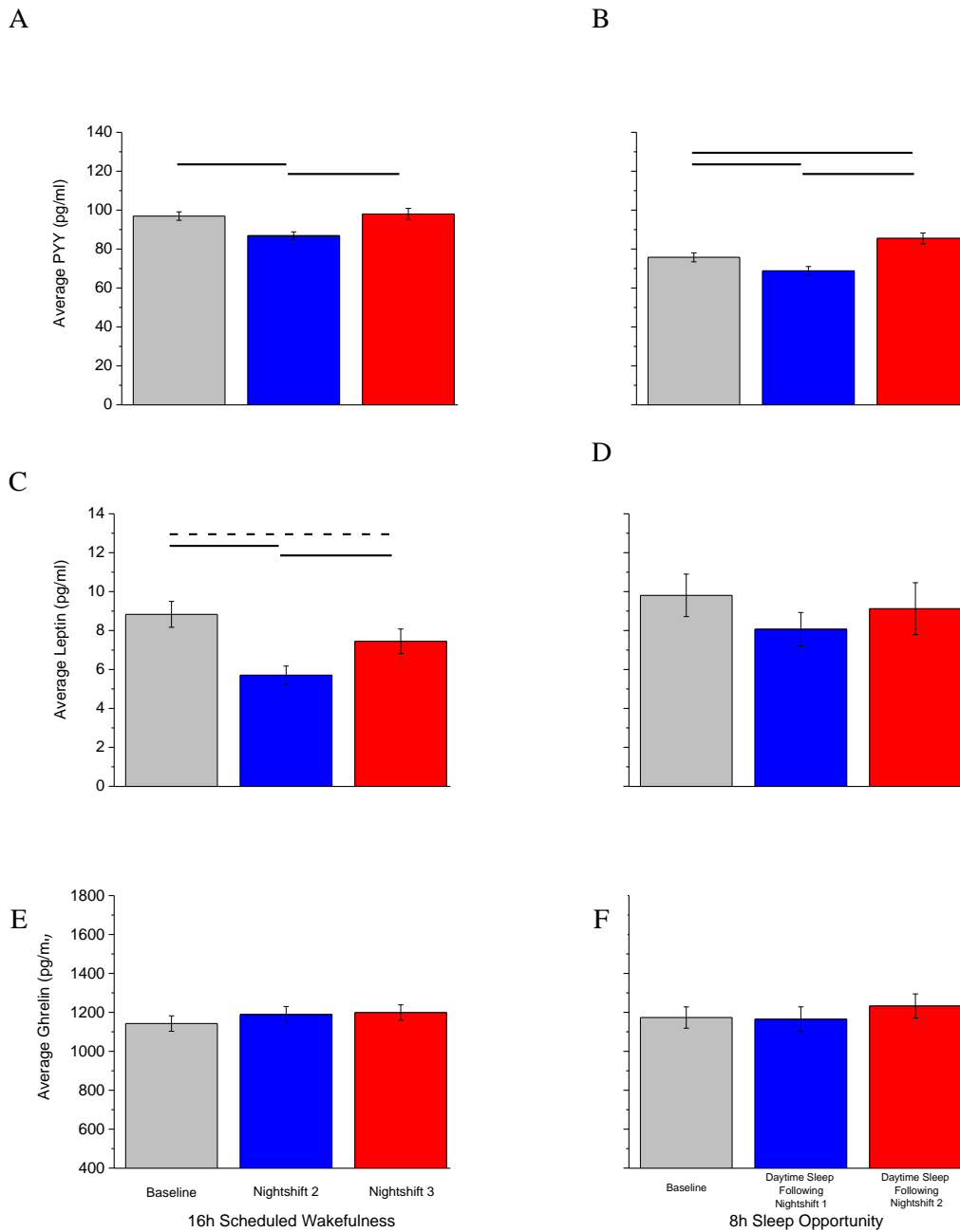
Fig. S4. Average satiety and hunger hormones during wakefulness (left) and scheduled sleep (right). Planned comparisons calculated by mixed model ANOVAS for study day. Solid lines represent significant differences ($p < 0.025$) and dashed lines indicate non-significant trends ($p = 0.06$) for points at the end of each line. Error bars are SEM. Average PYY levels decreased during wakefulness and scheduled sleep on nightshift 2 as compared to baseline, and increased during both wakefulness and scheduled sleep on nightshift 3 as compared to nightshift 2. Further, PYY levels increased above baseline during scheduled sleep on nightshift 3. Average leptin levels decreased during wakefulness on nightshift 2 and a non-significant trend ($p = 0.06$) for decreased levels on nightshift 3 as compared to baseline. Further, leptin levels increased during wakefulness on nightshift 3 as compared to nightshift 2. Leptin was similar between study days during scheduled sleep ($p > 0.13$). Average ghrelin was similar during wakefulness and scheduled sleep ($p > 0.25$).

CHAPTER 3

COGNITIVE IMPAIRMENT DURING THE TRANSITION TO NIGHT SHIFT WORK AND SUBSEQUENT NIGHT SHIFTS

Andrew W. McHill and Kenneth P. Wright Jr

Abstract:

Demands of modern society force many work operations into the late night when the internal

circadian timekeeping system is promoting sleep. The combination of disturbed daytime sleep

and circadian misalignment, as common in overnight shiftwork, decreases cognitive

performance, yet how these decreases in performance differ across subsequent nights of

shiftwork are still not fully understood. Therefore, the primary aim of this study was to examine

cognitive performance and behavior in a simulated nightshift work protocol across the hours of a

typical daytime shift, a first night shift with extended wakefulness, and two subsequent overnight

shifts. We tested the hypothesis that cognitive performance would be worse on the first nightshift

as compared to the baseline and subsequent nighttime working shifts, and that the performance

during nighttime working shifts would be reduced as compared to the baseline daytime shift.

Fifteen healthy adults (6 males) aged (26.13± 4.5 y; mean±SD) were studied in the 6-day

constant posture protocol (bedrest, head at 35º, dim light <5 lux). The baseline dayshift consisted

of daytime wakefulness and nighttime sleep at habitual timing. The first nightshift consisted of

wakefulness during the daytime, a 2h nap in the afternoon, and extended wakefulness through

the night to simulate transitioning to the first nightshift. Night shifts 2 and 3 consisted of daytime

sleep opportunities and wakefulness through the late afternoon and evening. Subjects completed

a series cognitive performance tests at times intended to simulate a 10h daytime or nighttime

work shift. Results show that working during the night increased subjective sleepiness and

decreased performance on the psychomotor vigilance task (i.e. decreased reaction time and

increased attentional lapses), Stroop color word task (decreased number of correct responses)

and mood. Sleepiness and mood increased from the first nightshift across subsequent nightshifts.

Our findings further demonstrate that nightshift work, regardless of whether it is the first

nightshift with extended wakefulness or subsequent nightshifts, decreases performance and mood as compared to the dayshift. Furthermore, we observed limited evidence of performance changes across subsequent nights of simulated night work; however we did observe improvements in subjective sleepiness and mood, potentially suggesting an end of study effect.

Introduction

In the modern 24h society, many occupations must often work during the night and sleep during the day when these actions are not promoted by the internal circadian timing system. This circadian misalignment, as common in shift work, has been correlated with disorders such as obesity (Antunes, et al. 2010), diabetes (Guo, et al. 2013), cardiovascular disease (Copertaro, et al. 2008), sleep disorders (Ohayon, et al. 2002), and potentially cancer (Wang, et al. 2011). Amongst the numerous negative health outcomes associated with shift work, disturbed sleep and impaired performance are the most reported complaints, with shift workers reporting higher incidence of sleep disruption than their daytime working counterparts (Åkerstedt 2003; Åkerstedt and Wright 2009; Drake, et al. 2004; Torsvall, et al. 1989). Furthermore, individuals who work permanent, rotating, or occasional nightshift work report "nodding off" during the shift at a higher incidence than those working during the day (Gold, et al. 1992) and have increased risk of accidents (Barger, et al. 2005; Smith, et al. 1994). These increases in reported sleepiness and decreases in performance amongst nightshift workers are the consequence of a combination of disturbed daytime sleep, circadian misalignment, and extended work hours, especially on the first night of a series of overnight shifts (Åkerstedt 1995; Lockley, et al. 2007).

Sleep and wakefulness are either promoted or inhibited by an interaction between the homeostatic pressure for sleep and the endogenous near 24h circadian clock located in the suprachiasmatic nucleus of the hypothalamus (Borbély 1982; Dijk and Czeisler 1994). During wakefulness, the homeostatic pressure for sleep builds in a near-linear fashion with duration of wakefulness, whilst the circadian system acts as an opponent process to counteract sleep pressure and promote wakefulness (Dijk and von Schantz 2005; Hull, et al. 2003). During the biological night, these two systems work in concert to promote a consolidated sleep episode (Borbély 1982;

Borbély and Achermann 1992; Dijk and Czeisler 1994). The alignment of these two physiological systems in this manner promotes sleep and wakefulness at optimal physiological times, whereas desynchrony between these two systems results in circadian misalignment, a state in which sleep promoted when the circadian system is promoting wakefulness and arousal (Markwald and Wright 2012) or when wakefulness occurs when the circadian system is promoting sleep. In most overnight shift workers, the circadian system is not able to adapt to the overnight schedule and circadian misalignment occurs (Dumont, et al. 2001; Folkard 2008; Sack, et al. 1992).

Transitioning from the dayshift to the first nightshift is a time of performance vulnerability in nightshift workers. These nightshifts are often preceded by short daytime naps and wakefulness for several hours prior to the start of the shift (Folkard 1992; Härmä, et al. 1989; Knauth, et al. 1980) Further, cognitive performance decreases with time awake (Dijk, et al. 1992) and the timing of the nightshift typically occurs during the circadian nadir in cognition (Dijk, et al. 1992; Frey, et al. 2004; Jewett and Kronauer 1999; Wright, et al. 2002). Many nightshift workers have reported wakefulness for greater than ~16h prior to starting their shift, resulting in ~24h of wakefulness at the end of an 8h shift (Folkard 1992; Rajaratnam and Arendt 2001). For perspective, at 17h of wakefulness after a typical morning waketime, cognitive psychomotor performance exhibits decrements comparable to those observed in an individual with a blood alcohol concentration of 0.05% (Dawson and Reid 1997). At 24h of sustained wakefulness, these decrements are equivalent to that of an individual with a blood alcohol concentration of 0.10% (Dawson and Reid 1997). Further, extended shifts have been found to exponentially increase the risk of accidents with a 12h shift having more than doubled the risk of the first 8h on shift (Folkard and Tucker 2003).

Whether performance decrements are most profound on the first nightshift or become more severe across multiple nightshifts remains unclear. Extended wakefulness on the first shift can extend beyond 12h, yet disturbed daytime sleep over subsequent shifts could accrue a sleep debt and further decrease performance. Findings from field studies show that risk of accidents increases and medical performance decreases across successive nightshifts (Dula, et al. 2001; Folkard and Lombardi 2006; Folkard and Tucker 2003), but also suggest that the first night is the most vulnerable to performance decreases due to the combination of extended wakefulness and circadian misalignment (Lockley, et al. 2004; Purnell, et al. 2002). Findings from controlled laboratory settings that simulated the first and subsequent nights of shiftwork show that the first night of shiftwork has more pronounced decrements as compared to the subsequent nightshifts (Lamond, et al. 2004; Santhi, et al. 2007). These conflicting findings require additional studies to better characterize the time course of performance decrements across subsequent nightshifts. Therefore the aim of this study was to investigate the changes in cognitive performance across a simulated shiftwork protocol that allowed for comparisons between a daytime work shift, a transition day with an afternoon nap prior to the first nightshift, and 2 subsequent nightshifts with daytime sleep episodes. We hypothesized that the cognitive performance during the nighttime working shifts would be reduced as compared to the day shift. Further, we hypothesized that cognitive performance would be worse on the first night shift with extended hours of wakefulness as compared to the baseline and two subsequent nighttime working shifts.

METHODS

Subjects

Fifteen healthy subjects (n= 6 males) aged 21-34 y (26.13± 4.5 y; mean±SD) with no reported diagnoses of any medical, psychiatric, or sleep disorders participated. Subjects gave written informed consent and study procedures were approved by the scientific advisory and review committee of the Colorado Clinical and Translational Sciences Institute, by the Colorado Multiple Institutional Review Board (IRB), and by the University of Colorado Boulder IRB. Subjects reported that they were not current smokers, pregnant, nor did they maintain habitual sleep durations of <7h or > 9.25 h. Subjects were deemed healthy by a physician administered physical exam, psychological tests and psychological interview with a trained clinician, blood chemistries (complete blood cell count and comprehensive metabolic panel), clinical electrocardiogram, and medication free status. Subjects reported no regular night work in the preceding year or crossing more than one time zone in the previous three weeks. Health screening and resting metabolic testing were conducted at the Sleep and Chronobiology Laboratory and at the Clinical Translational Research Center (CTRC) at the University Colorado Boulder.

Protocol

Ambulatory Monitoring

For one week prior to admission to the study, subjects were asked to maintain a self-selected sleep schedule of ~8h per night. The sleep schedule was verified with sleep-wakefulness logs, call-in bed and wake times to a time stamped voice-mailbox recorder, and wrist activity with light exposure recordings (Actiwatch-L, Mini Mitter Respironics, Bend, OR).

Drug use (including caffeine and nicotine) was proscribed for one week prior to in-laboratory testing and alcohol three days prior to and throughout the protocol. Drug use was determined by self-report and verified by urine toxicology at screening and by urine toxicology and a breath alcohol tester (Lifeloc Technologies Model FC10, Wheat Ridge, CO) upon laboratory admission.

In-Laboratory Protocol

Subjects lived in the CTRC at the University of Colorado Hospital for ~6 days under a constant posture protocol. Subjects were admitted to the laboratory ~7h prior to their habitual bedtime as determined from the week of 8h self-selected sleep schedules. At admittance, subjects were given cognitive performance batteries for practice to control for/remove the steep portion of the learning curve (Wertz, et al. 2006; Wright, et al. 1997; Wright, et al. 2006) and instructed that speed and accuracy are equally important in completing the tasks. Subjects were exposed to each task until achieving ≥90% on accuracy measures as verified by research staff. Polysomnography (PSG) was used to record an 8h baseline nighttime sleep episode at the subjects' habitual bedtime and to verify that subjects were free from sleep disorders by screening for sleep apnea and periodic limb movements based on standardized air flow, respiratory effort and leg EMG measurements. Following the sleep disorders screening night, subjects awoke on Day 2 (daytime shift) at habitual waketime as determined from the ambulatory monitoring week and maintained the constant posture protocol; ambient dim lighting (<1 lux in the angle of gaze, < 5 maximum) throughout scheduled wakefulness and 0 lux during scheduled sleep; seated semi-recumbent seated posture in a hospital bed (head raised to ~35 degrees during wakefulness and lowered to a supine position during scheduled sleep opportunities; and temperature maintained in the thermoneutral range (22-24º Celsius). Subjects were allowed 3 brief scheduled breaks to

obtain a meal and use the bathroom (a toilet <3m from the bed). Outside of scheduled breaks during wakefulness, subjects were given a urinal or bedpan to use if needed. The daytime shift consisted of 16h of wakefulness during the day and an 8h sleep opportunity during the night at habitual time. The transition to the first nightshift consisted of wakefulness at habitual waketime and a 2h afternoon sleep opportunity 6h prior to the first nightshift, a schedule common in many shiftworkers (Åkerstedt and Torsvall 1985; Härmä, et al. 1989). After the first nightshift, subjects were provided an 8h daytime sleep opportunity that began one hour after their habitual baseline waketime. The next two study days consisted of two days of nightshift work and daytime sleep (Fig. 1). After the last nightshift, subjects were provided an 8h recovery sleep opportunity on day 6 that was not PSG recorded and then were discharged from the hospital. Scheduled wakefulness and subject compliance during the constant posture routine was verified via continuous monitoring by research staff and electroencephalography (EEG) recordings.

Subjects completed a series of cognitive performance tests and questionnaires every two hours during wakefulness with the first set being given at two hours scheduled wakefulness and the last 2h prior to scheduled sleep.

Relative Clock Hour
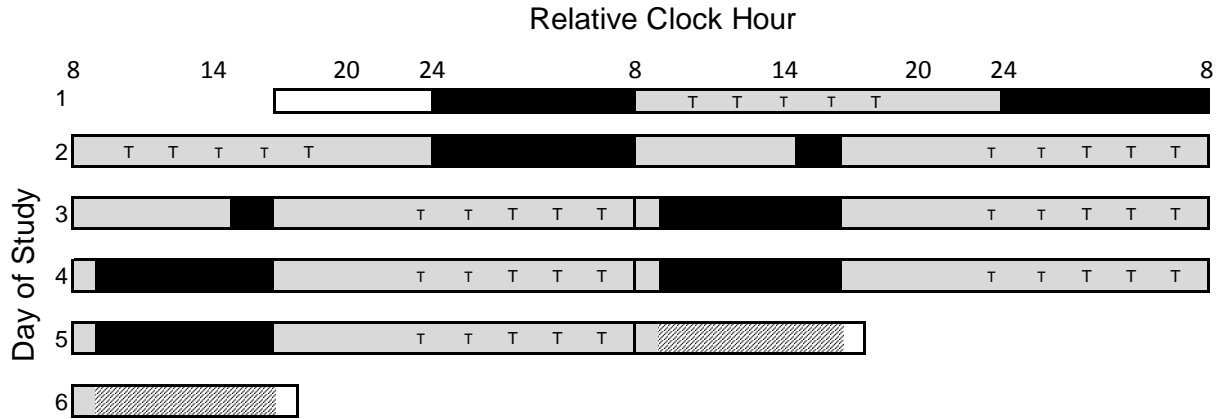
Figure 1. Study protocol. Time of day is plotted as relative clock hour with scheduled waketime arbitrarily assigned a value of 0800h and all other times referenced to this value. This protocol permitted subjects to sleep at their habitual circadian phase at baseline. Open bars represent scheduled performance practice tests in room light (<40lux). Black bars represent scheduled PSG recorded sleep. Hatched bars represent non-PSG recorded sleep. Shaded areas indicate scheduled wakefulness in dim light (<1 lux). Day 1 consisted of practice tests and a sleep disorders screen. Day 2 (dayshift) consisted of 16h of daytime wakefulness during the day with an 8h nighttime sleep opportunity at habitual time. Day 3 (transition and nightshift 1) consisted of 7h of wakefulness followed by a 2h nap and 15h of subsequent wakefulness and the last 10h was used to simulate nightshift 1. Days 4 (nightshift 2) and 5 (nightshift 3) consisted of an 8h daytime sleep opportunity and 16h of afternoon and nighttime wakefulness with the last 10h of the day representing the nightshift. Days are double plotted with successive days plotted both next to and beneath each other. T= time of cognitive test.

Performance Battery

Cognitive performance tasks were selected for their sensitivity to sleepiness, their validity, generalizability, and so that we could assess brain functions that are critical for work performance, decision-making, and public safety. We have conducted factor analyses on cognitive function during sleep deprivation and have found that different tasks provide unique information on cognitive impairments during sleep loss (i.e., not all tasks change in the same way or to the same degree) (Frey, et al. 2004). The computerized performance battery consisted of the Karolinska Sleepiness Scale (KSS), Psychomotor Vigilance Task (PVT), Stroop Color Word Test (STROOP), Calculation Performance Task (ADD), and a series of Visual Analog Scales (VAS). The KSS is a questionnaire that assesses subjective sleepiness by asking the

subject to rate on a scale of 1-9 how sleepy they feel at the moment of the test (Åkerstedt and Gillberg 1990). The PVT is a 10-min reaction time (RT) test that assesses sustained vigilance via presenting a millisecond number counter stimulus and determining RT with a button press (Dinges and Powell 1985; Van Dongen, et al. 2003). The STROOP color word test assesses accuracy, cognitive speed, and the inhibitory control component of executive function. This task requires subjects to respond by a button press indicating the color of the text stimulus presented on the computer screen. The text are colored such that the word is either the same as the color (e.g. the word "blue" is colored blue—congruent) or not (e.g. the word "blue" is colored green—incongruent) or non-words (colored XXXX—neutral). Inhibitory control components of executive function are calculated by subtracting the median RT of correct responses for neutral from incongruent stimuli. The ADD task assesses the accuracy and speed of mathematical calculations (Wertz, et al. 2006; Wright, et al. 2002; Wright, et al. 2006) by presenting the subject with randomly generated pairs of 2-digit numbers (e.g. 15 +26). The VAS assess mood by prompting the subject to identify on a 100mm horizontal line how the they feel at that moment, with each end of the line labeled with the extremes of a subjective continuum (e.g., words or phrases such as "very alert" and "very sleepy")(Wright, et al. 2002). Of the seventeen VAS items measured, we chose to analyze alertness, clearheadedness, sadness, and relaxed as we have shown three of the four of these measures to be sensitive to sleep deprivation and circadian misalignment (McHill, et al. 2014). In total, the performance battery lasted approximately 35 minutes.

Polysomnography Recordings

PSG recordings were obtained with Siesta digital sleep recorders (Compumedics USA Ltd, Charlotte, NC) from C3-A2, C4-A1, O1-A2, F3-A2, right and left electrooculogram (EOG), chin electromyogram (EMG), and electrocardiogram (ECG). Sleep was manually scored in 30-second epochs according to standard guidelines from brain region C3-A2 (Rechtschaffen and Kales 1968). If the C3-A2 trace contained artifact the C4-A1 trace was used to determine the sleep stage.

Data Analysis

Performance measures were aligned such that data were compared across a 10h "work day" during simulated daytime and nighttime shiftwork. Thus, the first five performance batteries during the dayshift (relative timing 1000-1800h for an individual with habitual waketime of 0800h) and the last five performance tests on the first through third nightshifts (relative timing 2300-0700) were analyzed.

The PVT was analyzed as reciprocal transformation of the median RT, reciprocal transformation of the 10 percent slowest RT, the 10 percent fastest RT, and for lapses in attention (RT ± 500msec) (Jung, et al. 2011; Van Dongen, et al. 2003). The STROOP color word task was analyzed as the overall number correct for congruent presentations incongruent presentations, neutral presentations, the median RT for each of the overall correct responses (congruent, incongruent, and neutral) and as the difference in median correct RT between incongruent and neutral responses to asses inhibitory control. The ADD task was analyzed as number of problems attempted and the number of equations answered correctly during the 4-minute task.

Sleep data were analyzed for sleep opportunities after the dayshift (habitual bedtime baseline), and after the first and second nightshifts (daytime sleep). Sleep efficiency (SE) was

binned per hour of each sleep opportunity across each sleep episode. Detailed sleep architecture analyses are provided in Chapter 2 of this dissertation. Average performance tasks over the work shift and SE during the sleep opportunities were analyzed using Mixed Model ANOVA with work shift and hours into work shift as fixed factors and subject as a random factor using STATISTICA, version 10.0 (StatSoft Inc, Tulsa, OK). Planned comparisons were conducted for each 2h test and SE comparisons using dependent t-tests.

Results

Sleep Efficiency

A significant interaction between sleep opportunity and hours into sleep opportunity was observed (Fig 2. $p<0.01$). Planned comparisons revealed significantly higher SE during the daytime sleep opportunities for the first 3h of the sleep episodes, and a higher SE for the nighttime sleep opportunity for the last 2h of the sleep episodes (Fig. 2, $p<0.05$).
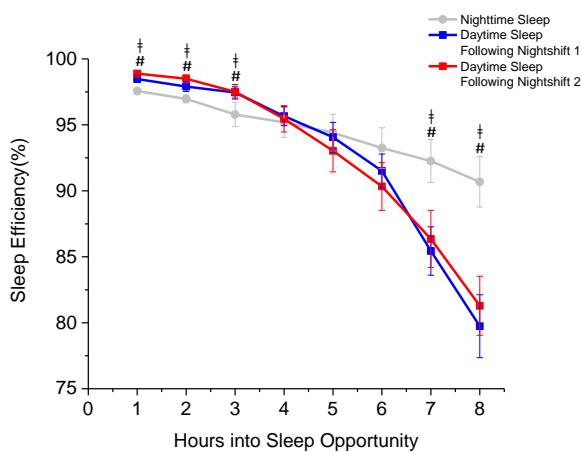
Figure 2. Hourly sleep efficiency during the baseline night and daytime sleep opportunities. Planned comparisons calculated with dependent t-test ($p<0.05$). # indicates differences between dayshift and nightshift 2, ‡ indicates differences between dayshift and nightshift 3. Error bars are SEM.

Subjective Sleepiness

A main effect for work shift for average KSS scores (Fig. 3 $p < 0.0001$) and significant interactions between work shift and hours into the work shift for KSS scores were observed, (Fig 3A, B, $p<0.05$). Planned comparisons revealed significantly higher sleepiness ratings on all night shifts as compared to the dayshift, and higher sleepiness ratings on nightshift 1 compared to nightshift 3 (Fig. 3A, $p<0.05$). Differences in sleepiness between the dayshift and the nightshifts were observed for hours 6-10 of the work shift (Fig. 3B, $p<0.05$).
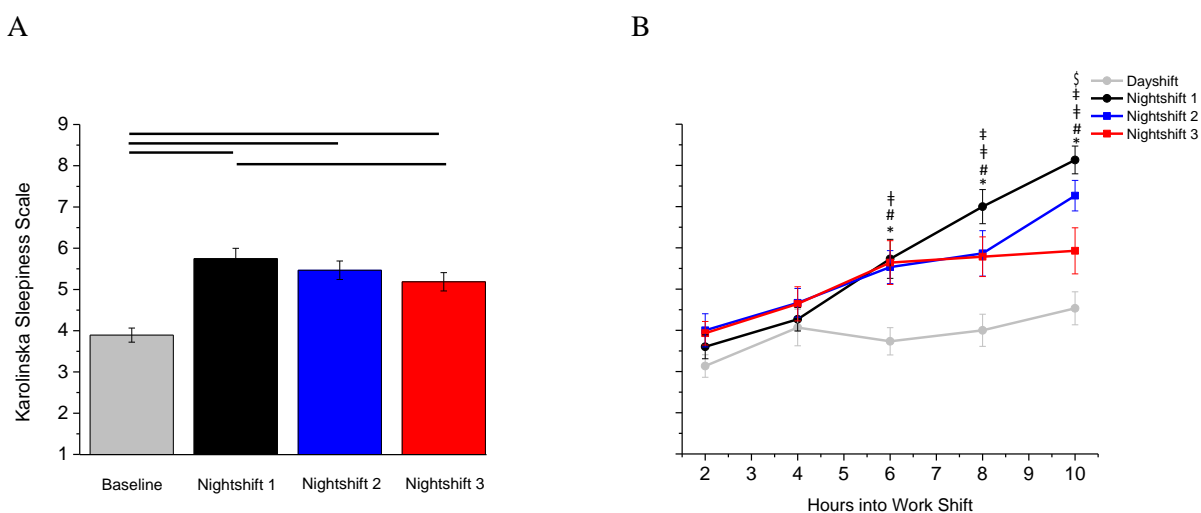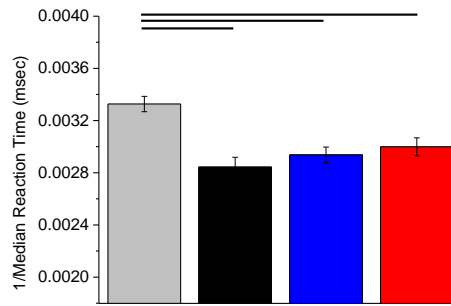

Figure 3. KSS. Left panel shows average performance for each work shift. Solid lines represent significant differences for points at the end of each line ($p<0.05$). Right panel, shows performance every 2h across the work shift. * indicates differences between the dayshift and the nightshift 1, # indicates differences between the dayshift and nightshift 2, ⸸ indicates differences between the dayshift and third nightshift, † indicates differences between the nightshift 1 and nightshift 2, ‡ indicates differences between nightshift 1 and nightshift 3, $ indicates differences between nightshift 2 and nightshift 3. Error bars are SEM.

Pyschomotor Vigilance Task

A main effect for work shift for average PVT outcomes (Fig. 4A, 4C, 4E, 4G; all $p<0.0001$) and significant interactions for work shift and hours into the work shift for all PVT outcomes were observed (Fig. 4B, D, F, H, $p<0.01$). Planned comparisons revealed significantly slower 1/median RT, slowest RT, and fastest RT (Fig. 4A, C, E, $p<0.05$) and significantly increased lapses of attention (Fig. 4G, $p<0.05$) on all overnight shifts as compared to the dayshift. A non-significant trend for increased 1/slowest RT was also found between the nightshift 1 and nightshift 3 (Fig. 4C, $p=0.07$). Significant decreases in PVT performance were observed on nightshifts for hours 6-10 of the work shift for all PVT measures (Fig. 4B, 4D, 4F, 4H) compared to the dayshift. Furthermore, 1/median RT performance was worse at the beginning of the nightshifts and fastest RT at 4h into work shift as compared to dayshift (Fig 4B, 4F).
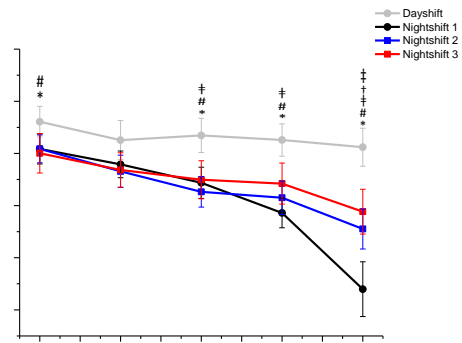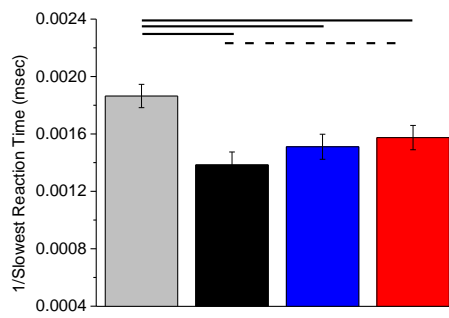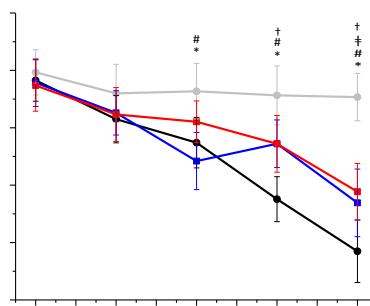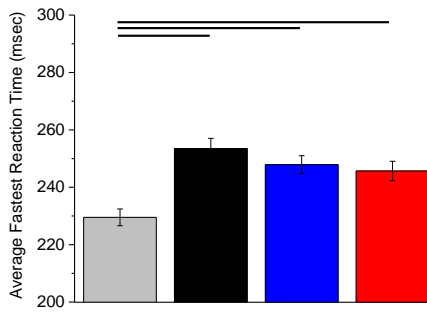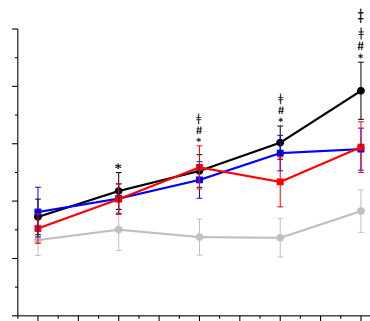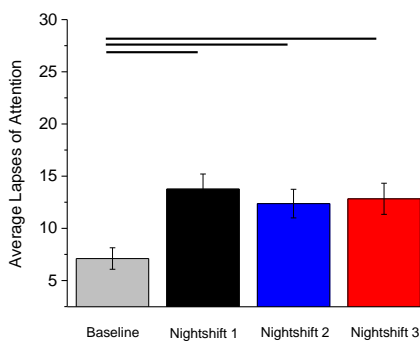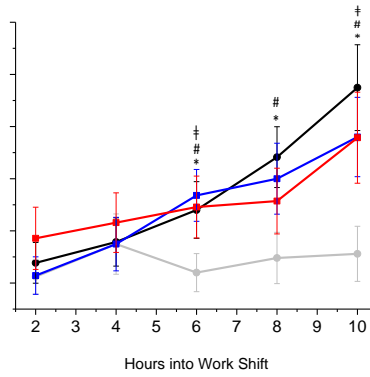
Figure 4. PVT reciprocal median RT, reciprocal 1/slowest RT, mean fastest RT, and mean lapses. Left panel shows average performance for each work shift. Solid lines represent significant (p<0.05) differences and dashed lines indicate a non-significant trend (p=0.07) for points at the end of each line. Right panel, shows performance every 2h across the work shift. * indicates differences between the dayshift and the nightshift 1, # indicates differences between the dayshift and nightshift 2, ǂ indicates differences between the dayshift and third nightshift, † indicates differences between the nightshift 1 and nightshift 2, ‡ indicates differences between nightshift 1 and nightshift 3. Error bars are SEM.

STROOP Color Word Task

There was a significant main effect of work shift and hours into working shift for the number of correct responses for the congruent, neutral, and incongruent presentations (Fig. 5A-F, p<0.05). Planned comparisons revealed significantly decreased average congruent correct, incongruent correct, and neutral correct on all night shifts as compared to the dayshift (Fig. 5A, C, D, p<0.05) with a significant increase in neutral number correct between the nightshift 1 and nightshift 3 (Fig. 5E, p<0.05). Hourly number correct were generally decreased during the nightshifts as compared to the dayshift (Fig. 5B, D, E). There were significant interactions for work shift and hours into the work shift for congruent correct median RT and incongruent correct median RT and a significant main effect of hours into work shift for neutral correct median RT (Fig. 6A-F, p<0.05). Planned comparisons revealed a decrease in neutral correct median RT from nightshift 2 to nightshift 3 (Fig. 6E, p<0.05), and a non-significant trend for increased average neutral correct median RT between the dayshift and nightshift 2 (Fig. 6E, p=0.07) and decreased congruent correct median RT and neutral correct median RT between nightshift 1 and nightshift 3 (Fig. 6A, E, p=0.07, 0.09, respectively). Significant decreases in correct median RT were observed on nightshifts for hour 2 of the work shift for average congruent correct median RT and average incongruent correct median RT and hours 8-10 for average neutral correct median RT as compared to dayshift (Fig. 6B, D, F, p<0.05). There was a

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significant main effect for work shift for incongruent-neutral correct median RT (Fig. 7A, $p<0.05$). Planned comparisons revealed a decrease in incongruent-neutral correct median RT between the dayshift and nightshift 1 (Fig. 7A, $p<0.05$) and a non-significant trend for decreased incongruent-neutral correct median RT between the dayshift and nightshift 2 (Fig. 4F, $p=0.08$). Further, significant decreases in incongruent-neutral median RT were observed on nightshifts for hours 2-4 of the work shift as compared to dayshift (Fig. 7B, $p<0.05$).
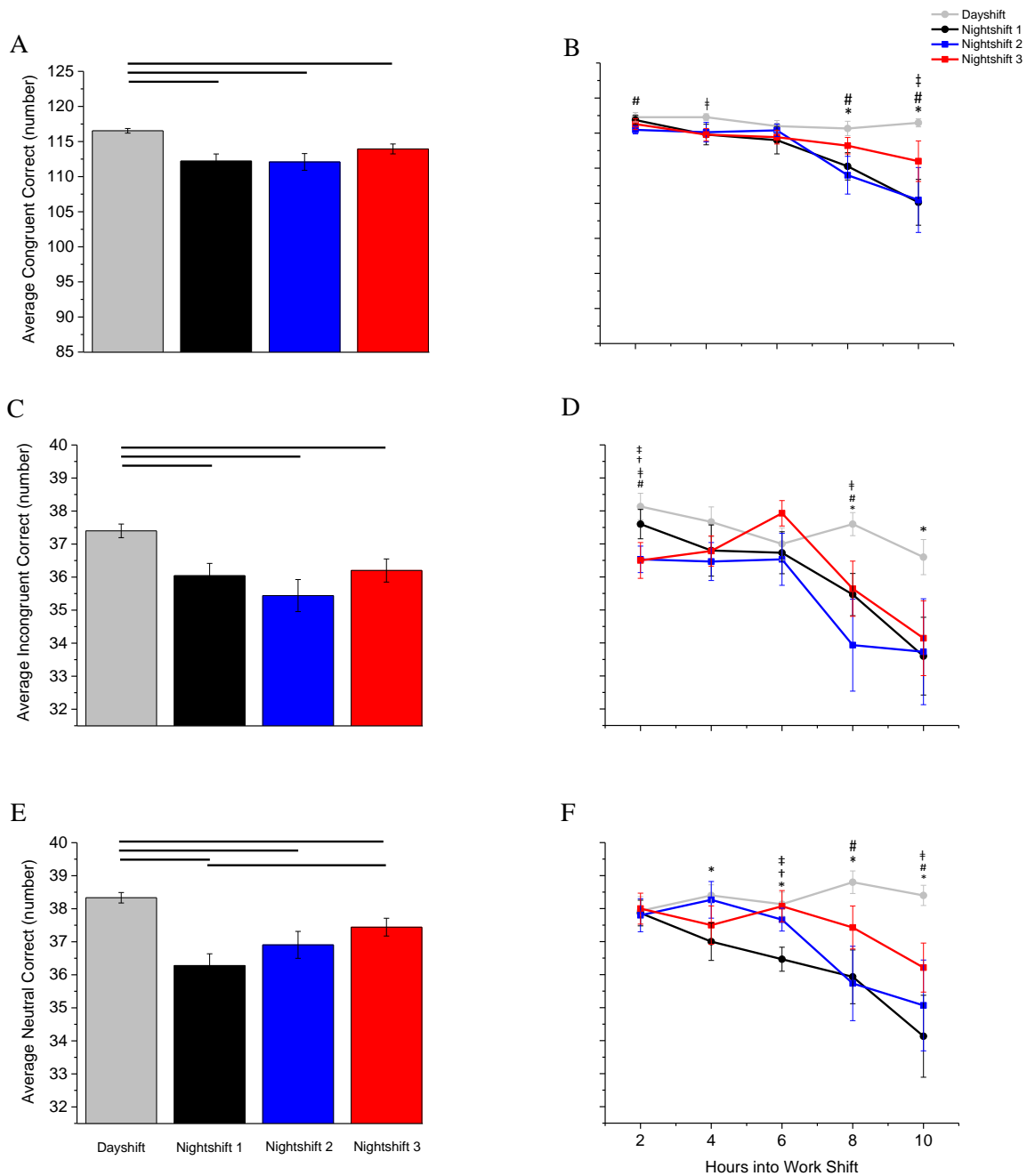
Figure 5. STROOP color word task average congruent correct, incongruent correct, and neutral correct. Left panel shows average number correct for each work shift. Solid lines represent significant differences and for points at the end of each line (p<0.05). Right panel, shows performance every 2h across the work shift. * indicates differences between the dayshift and the nightshift 1, # indicates differences between the dayshift and nightshift 2, ‡ indicates differences between the dayshift and third nightshift, † indicates differences between the nightshift 1 and nightshift 2, ‡ indicates differences between nightshift 1 and nightshift 3. Error bars are SEM.

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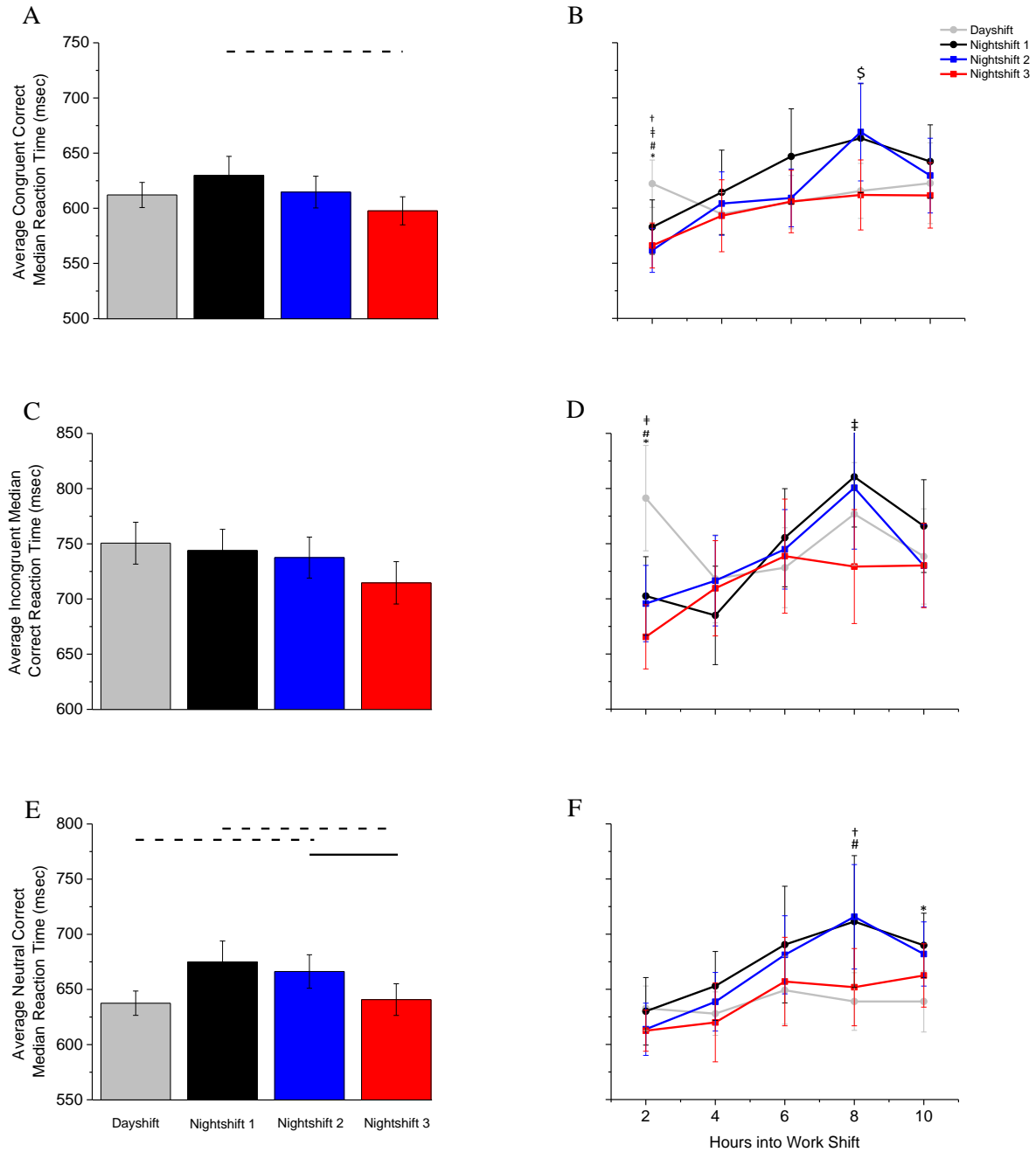
Figure 6. STROOP color word task average congruent correct median RT, incongruent correct median RT, and neutral correct median RT. Left panel shows average number correct for each work shift. Solid lines represent significant (p<0.05) differences and dashed lines indicate a non-significant trend (p<0.1) for points at the end of each line. Right panel, shows performance every 2h across the work shift. * indicates differences between the dayshift and the nightshift 1, # indicates differences between the dayshift and nightshift 2, ‡ indicates differences between the dayshift and third nightshift, † indicates differences between the nightshift 1 and nightshift 2, ‡ indicates differences between nightshift 1 and nightshift 3, $ indicates differences between nightshift 2 and nightshift 3. Error bars are SEM.
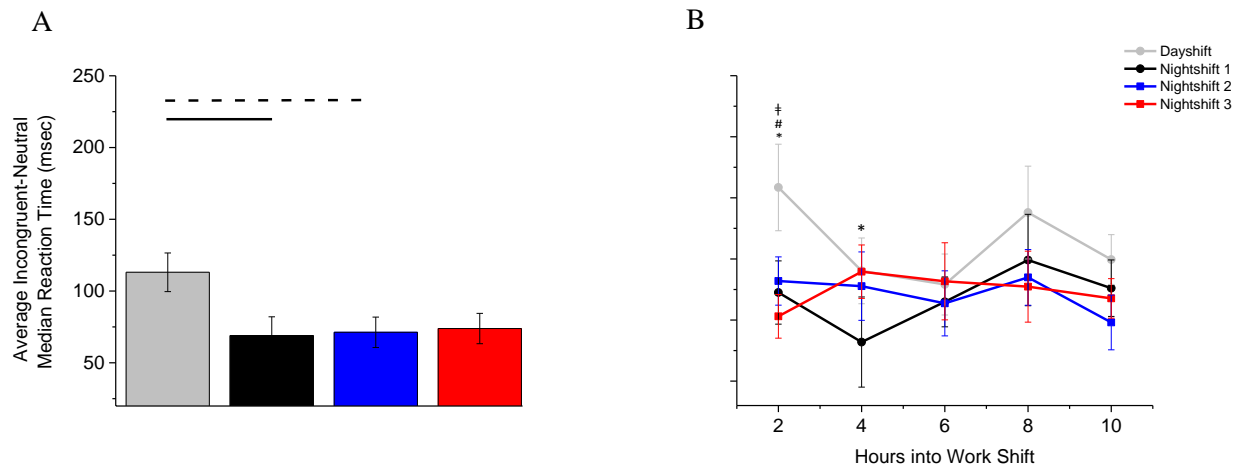
Figure 7. STROOP color word task average incongruent-congruent correct median RT. Left panel shows average number correct for each work shift. Solid lines represent significant (p<0.05) differences and dashed lines indicate a non-significant trend (p<0.08) for points at the end of each line. Right panel, shows performance every 2h across the work shift. * indicates differences between the dayshift and the nightshift 1, # indicates differences between the dayshift and nightshift 2, ‡ indicates differences between the dayshift and third nightshift, † indicates differences between the nightshift 1 and nightshift 2, ‡ indicates differences between nightshift 1 and nightshift 3, $ indicates differences between nightshift 2 and nightshift 3. Error bars are SEM.

ADD Task

Significant main effects of work shift and hours into working shift were observed for both the number of equations attempted and number of equations answered correctly (Fig. 8A-D, $p<0.05$). Planned comparisons revealed increases in the number of attempted and correct responses between nightshift 3 and all other work shifts (Fig. 8A, C, $p<0.05$). Significant increases were observed on nightshifts at hour 2 for both number attempted and number correct compared to baseline (Fig. 8B, D, $p<0.05$) and number correct was on nightshift 1 were decreased compared to nightshift 3 and dayshift. Furthermore, number attempted on nightshift 3 was increased at hour 8 as compared to dayshift (Fig. 8B, $p<0.05$)

Figure 8. Average ADD task and hourly differences across circadian misalignment. Left panel shows average performance for each work shift. Right panel, shows performance every 2h across the work shift. * indicates differences between the dayshift and the nightshift 1, # indicates differences between the dayshift and nightshift 2, ǂ indicates differences between the dayshift and nightshift 3, † indicates differences between the nightshift 1 and nightshift 2, ‡ indicates difference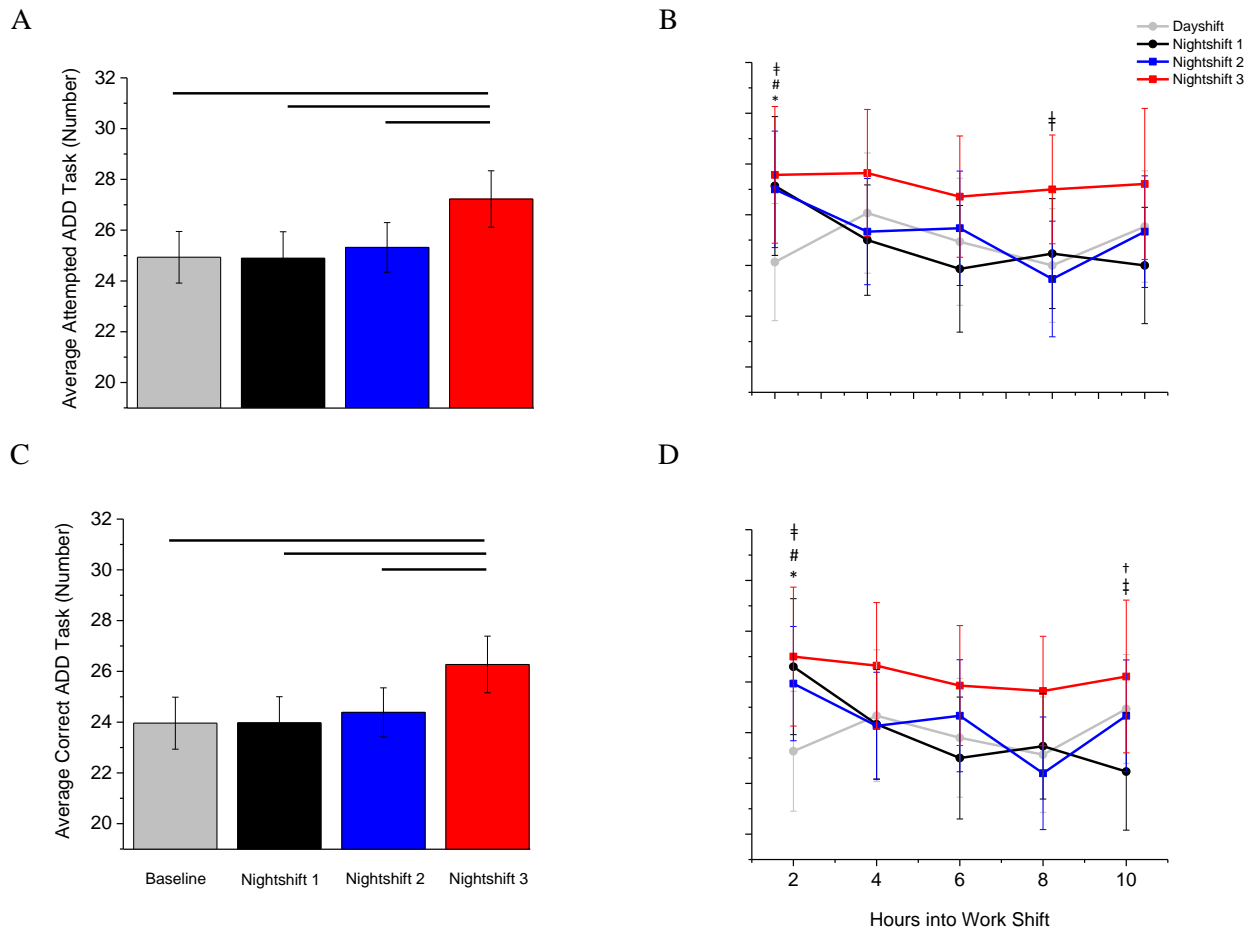s between nightshift 1 and nightshift 3. Error bars are SEM. Both the number of equations attempted and correct increased across study days.

Mood

Significant interactions for work shift and hours into the work shift for clear headedness and alertness ratings were observed (Fig. 9A-D, $p<0.01$) and a significant main effect for work shift for sadness ratings was observed (Fig. 9E, $p<0.05$). Planned comparisons revealed significant decreases in clear headedness and alertness ratings between all nightshifts and the dayshift, with increases between nightshift 1 and nightshifts 2 and 3 (Fig. 9A, C $p<0.05$). Further, planned comparisons revealed a significant increase in sadness rating between the dayshift and nightshift 3, (Fig. 9E, $p<0.001$) and a non-significant trend for increased sadness ratings between the nightshift 1 and nightshift 3 (Fig. 9E, $p=0.07$). Significant decreases in clear headedness and alertness ratings were observed on the nightshifts for most of the hours into the work shift as compared to the dayshift (Fig. 9B, D). Significant decreases in sadness ratings were observed at hour 2 between nightshift 3 and dayshift (Fig. 9F). No significant difference was observed in relaxed ratings across work shifts (Fig. 9G, $p>0.45$), however a significant decrease in relaxed ratings was observed at hour 4 between nightshift 1 and dayshift (Fig. 9H, $p<0.05$).
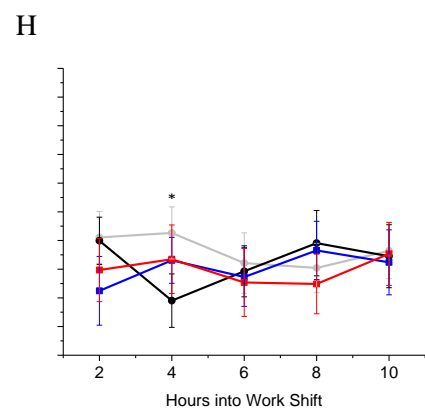
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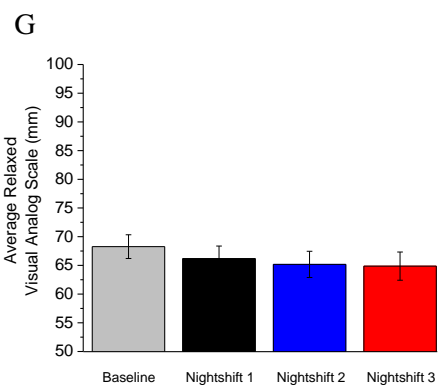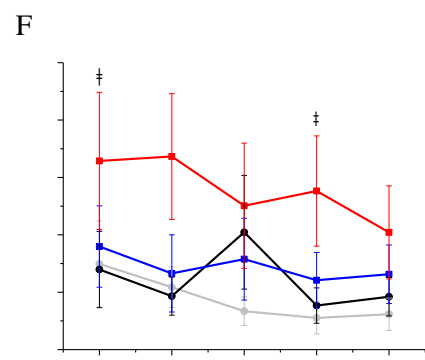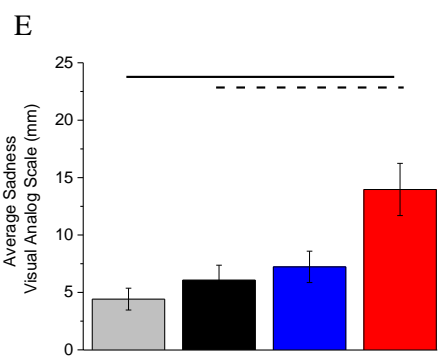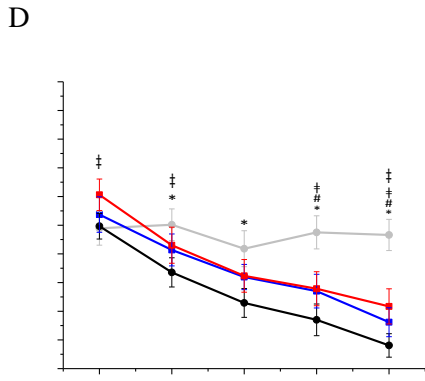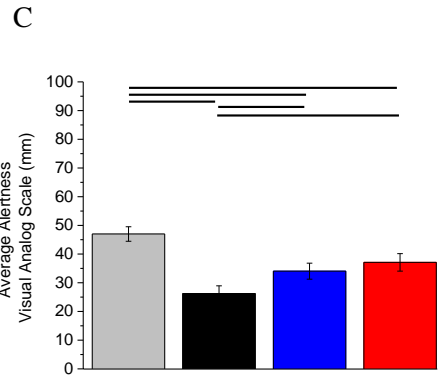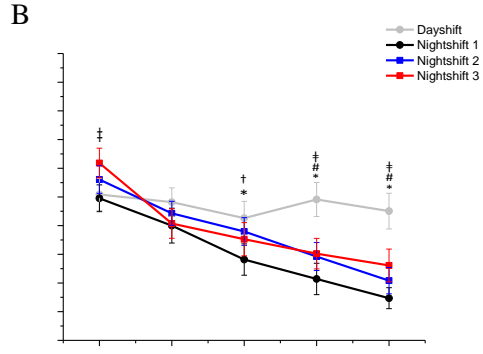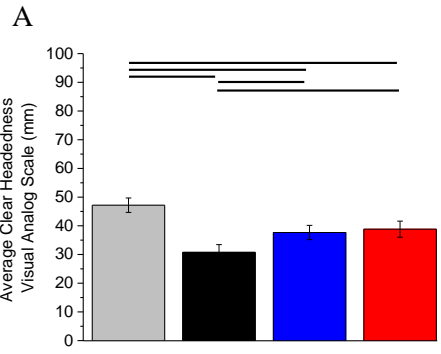
Figure 9.VAS scores. Left panel shows average performance for each work shift. Solid lines represent significant (p<0.05) differences and dashed lines indicate a non-significant trend (p=0.07) for points at the end of each line. Right panel, shows performance every 2h across the work shift. * indicates differences between the dayshift and the nightshift 1, # indicates differences between the dayshift and nightshift 2, ǂ indicates differences between the dayshift and third nightshift, † indicates differences between the nightshift 1 and nightshift 2, ‡ indicates differences between nightshift 1 and nightshift 3. Error bars are SEM. Note that the y-axis for Relaxed is one half and Sad is one quarter of Clear headedness and Alertness.

Discussion

Findings from the current study further our knowledge of how sleepiness and cognitive performance change across the transition to, and during subsequent nights of working a simulated nightshift. Importantly, we found that sleeping during the daytime decreased SE in the last two hours of the daytime sleep opportunity and that working during the night increased subjective sleepiness, decreased the median, slowest 10%, and fastest 10% RT and increased number of lapses of attention on the PVT, decreased the number of correct responses on the STROOP color word task, and decreased subjective alertness and clear headedness ratings when compared to working a dayshift. Further, we observed that nightshift 1 as compared to subsequent nightshifts showed lower KSS subjective sleepiness ratings, but worse mood ratings and worse RT and accuracy performance scores. The decrements of performance, particularly decreases in RT and increases in lapses of attention, could help to explain increased risk for accidents and injury when work operations occur during the night (Barger, et al. 2005; Gold, et al. 1992; Smith, et al. 1994; Vila 2006). However, not all performance worsened during the night shift schedule as we observed improvements in the inhibitory control component of executive function for the STROOP task and in mathematical addition performance on nightshift

1 and nightshift 3, respectively, compared to the dayshift indicating evidence of learning of some tasks during nightshift work.

Sleep and Sleepiness

Consistent with prior findings, we found that the daytime sleep episode was disturbed during a shiftwork schedule (McHill, et al. 2014; Wright Jr, et al. 2013; Åkerstedt 2003; Åkerstedt, et al. 2008; Åkerstedt and Wright Jr 2009). Further, we found that the disruptions of sleep occur in the later portion of the daytime sleep episode, and that SE is improved at the beginning of the daytime episode relative to the baseline nighttime sleep episode. The decreased SE in the latter portion of the daytime sleep opportunities may be a consequence of the exponential decrease in sleep pressure during sleep and the increased circadian promotion of arousal at this time, thereby making it difficult to maintain sleep (Borbély 1982; Dijk and Czeisler 1995; Wright, et al. 2013). This time course for disturbed latter portions of daytime sleep in shiftworkers may lead to accumulated sleep debt over successive nights of shiftwork and negatively impact performance concurrently with circadian misalignment (Tilley, et al. 1982). One strategy to improve sleep in shiftworkers is a combination of an anchor sleep episode after the work shift and a nap prior to starting the nightshift. Findings from a combined field and laboratory study show that an anchor sleep episode post the overnight shift for ~5-6h, combined with an evening nap prior to the shift and caffeine during the shift, helps to improve psychomotor performance during the nightshift (Schweitzer, et al. 2006). While our data does not specifically address this strategy, we did observe decreases in SE beginning at ~6h into the

scheduled daytime sleep opportunity, suggesting that an anchor daytime sleep of this length would improve overall SE.

Concurrent with sleep disruption, sleepiness is a common complaint amongst individuals working overnight shiftwork, particularly during extended wakefulness (Åkerstedt and Wright 2009) or if diagnosed with shift work disorder (Drake, et al. 2004; Wright, et al. 2013). The increases in KSS scores observed in the present study during the nightshift are in agreement with increased sleepiness during the transition to nightshift work and during subsequent nightshifts. KSS scores were found however, to significantly decrease on nightshift 3 as compared to nightshift 1, which may indicate some adaption to the perception of sleepiness though sleepiness ratings did not return to baseline levels and as noted, performance for most tasks were worse on the nightshift.

Cognitive Performance

Our primary outcome for performance was median RT and the number of lapses on the PVT, two highly sensitive measures to sleep loss (Lim and Dinges 2008). We also examined the 10% slowest RT and the 10% fastest RT to examine how subject's performance changed in the lapse domain and optimum response times domain, respectively. Compared to the dayshift, PVT RT measures were slower during nightshift work. These findings show that psychomotor performance is decreased not only on nightshift 1 with extended wakefulness, but also remains decreased on subsequent nightshifts. Contrary to our hypothesis, PVT performance did not significantly differ across nightshift 1 to subsequent nightshifts. In contrast to our findings, similar laboratory studies have found performance improvements across successive nightshifts. Santhi and colleagues found that PVT performance, specifically lapses, improved on the average

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of two subsequent nights of shiftwork in laboratory settings (Santhi, et al. 2007), and Lamond and colleagues observed decreased RT across one week of simulated overnight shiftwork (Lamond, et al. 2004). Our findings may differ from previous laboratory studies due to several reasons. Previous studies simulating the first night of shiftwork did not allow for a daytime sleep opportunity prior to the first nightshift, whereas the current study included a 2h afternoon nap prior to nightshift 1, a schedule common in many shiftworks (Härmä, et al. 1989a; Åkerstedt and Torsvall 1985). This nap opportunity may have attenuated performance decrements observed on nightshift 1 and made that shift more similar to subsequent shifts. Further, we compared performance only during hours of the work shift, so as to capture differences that would occur when attention would be necessary while on the job.

The STROOP color word task was used to assess accuracy and speed of cognition as well as the inhibitory control component of executive function. We found that the number of correct responses during the test decreased during circadian misalignment, regardless of the type of stimulus presented (e.g. congruent, incongruent, neutral), with non-significant changes in the speed of correct responses suggesting that subjects maintained speed at the cost of accuracy on the nightshift. The inhibitory control of executive function, however, improved between the dayshift and nightshift 1, suggesting continued learning of the task. Similarly, performance on the ADD task improved during night shift work being higher on nightshift 3 compared to other shifts. This is in contrast to prior findings that indicated mathematical performance worsened during sleep loss and during weeks of circadian misalignment (Johnson, et al. 1992; Wright, et al. 2002; Wright, et al. 2006). Prior to the sleep disorder screening sleep opportunity, subjects were given frequent practice tests to eliminate steep portions of the learning curve. All subjects achieved ≥ 90% on all tasks prior to data collection. It is possible that the acute circadian

misalignment permitted continued learning on these tasks, but that more chronic circadian misalignment may worsen such performance.

Consistent with the literature, alertness and clear headedness ratings decreased and sadness increased during sleep deprivation and circadian misalignment (Dinges DF, et al. 1997; Lieberman, et al. 2005; McHill, et al. 2014). Furthermore, clear headedness and alertness levels increased across nightshifts as compared to nightshift 1. As the subjects in the current study were not blinded to study length or time cues and as we did not have a day working control condition, an end of study effect on any of the observed improvements on the last day in the study cannot be ruled out.

Summary

Understanding the time course of performance decrements across subsequent days of nightshift work is vital in development of evidenced-based countermeasure strategies. Our findings contribute to the existing knowledge and further demonstrate that working at adverse circadian times, regardless of whether it is the first nightshift with a nap and extended wakefulness or subsequent nightshifts, decreases performance and mood as compared to the working during the day. Contrary to other simulated laboratory night shiftwork protocols (Lamond, et al. 2004; Santhi, et al. 2007) and field studies (Dula, et al. 2001; Folkard and Lombardi 2006; Folkard and Tucker 2003), we observed little evidence of changes in performance across subsequent nights of shiftwork. Thus, our findings are consistent with findings that a nap prior to the first nightshift helps to attenuate performance decrements of extended wakefulness (Dinges, et al. 1987; Härmä, et al. 1989a; Schweitzer, et al. 2006), but

further countermeasures such as other wakefulness promoting agents (e.g. caffeine) or prophylactic naps may be necessary to combat fatigue on subsequent nightshifts.

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CHAPTER 4

EFFECTS OF CAFFEINE ON SKIN AND CORE TEMPERATURES, ALERTNESS AND RECOVERY SLEEP DURING CIRCADIAN MISALIGNMENT

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Abstract: Caffeine promotes wakefulness during night shift work, although it also disturbs subsequent daytime sleep. Increased alertness by caffeine is associated with a higher core body temperature (CBT). A lower CBT and a narrow distal-to-proximal skin temperature gradient (DPG) have been reported to be associated with improved sleep, yet whether caffeine influences the DPG is unknown. We tested the hypothesis that caffeine during nighttime total sleep deprivation would reduce the DPG, increase CBT and alertness, and disturb subsequent daytime recovery sleep. We also expected that a greater widening of the DPG prior to sleep would be associated with a greater degree of sleep disturbance. Thirty healthy adults (9 females) aged (21.6 ± 3.5y) participated in a double-blind, 28h modified constant routine protocol. At 23h of wakefulness, participants in the treatment condition (n=10) were given 2.9 mg/kg caffeine, equivalent to ~200mg or two espressos for a 70 kg adult, 5h before a daytime recovery sleep episode. Throughout the protocol, core and skin body temperatures, DPG, sleep architecture, and subjective alertness and mood were measured. Prior to sleep, caffeine significantly widened the DPG, increased CBT, alertness and clear-headedness ($p<0.05$). Caffeine also disturbed daytime recovery sleep ($p<0.05$). Increased CBT and a wider DPG prior to sleep were associated with a longer latency to sleep and a wider DPG was associated with disturbed recovery sleep (i.e., increased WASO and stage 1 sleep and decreased sleep efficiency, and SWS) ($p<0.05$). A widening of the DPG following nighttime caffeine may represent a component of the integrated physiological response by which caffeine improves alertness and disturbs subsequent daytime recovery sleep. Furthermore, our findings highlight that sleep disturbances associated with caffeine consumed near the circadian trough of alertness, are still present when daytime recovery sleep occurs 5h or approximately one-half life later.

INTRODUCTION

Sleep, circadian and thermoregulatory systems are highly integrated. Associations

between the circadian core body temperature (CBT) rhythm and circadian rhythms in

performance and sleep propensity have long been acknowledged (Kleitman and Jackson, 1950;

Czeisler et al., 1980; Wright et al., 2002). Humans typically initiate sleep on the descending limb

of the circadian CBT rhythm and subsequently initiate wakefulness on the rising limb of the

circadian CBT rhythm (Czeisler et al., 1980; Zulley et al., 1981; Campbell and Broughton, 1994;

Kräuchi and Wirz-Justice, 2001). Additionally, during circadian desynchrony, sleep propensity is

strongest when CBT is maximally declining and near its circadian low (Campbell and

Broughton, 1994; Dijk and Czeisler, 1994). Findings from the literature suggest that the

circadian decline in CBT is in part the result of heat loss via the periphery (Smolander et al.,

1993; Kräuchi et al., 2000). The distal-to-proximal skin temperature gradient (DPG) is an

indirect measurement of heat loss where temperature at distal skin sites (e.g. hands or feet) is

subtracted from temperature at proximal skin sites (e.g., subclavicular area, head, or stomach).

Proximal skin temperature is located near the core of the body and is thus typically warmer than

distal skin temperature. A larger difference between proximal and distal skin temperature is

described as a wider DPG, whereas when distal skin temperature warms and becomes closer to

proximal skin temperature, the DPG is described as more narrow. Narrowing of the DPG is

associated with peripheral vasodilation that allows increased blood flow to distal skin sites,

promoting heat loss by way of cutaneous vascular structures (i.e. arteriovenous anastomoses and

capillaries) (Krogstad et al., 1995). The DPG shows a circadian rhythm such that prior to

habitual sleep time, the DPG narrows whereas prior to habitual waketime the DPG widens

(Smolander et al., 1993; Kräuchi and Wirz-Justice, 1994; Kräuchi et al., 1997). The change in

DPG is reported to be a better predictor for sleep onset latency (SOL) than melatonin onset,

subjective sleepiness ratings, or rate of CBT decline (Kräuchi et al., 1999; Kräuchi et al., 2000).

Furthermore, manipulation of skin temperature leads to changes in alertness and sleep (Raymann et al., 2005; Raymann and Van Someren, 2007; Raymann et al., 2008).

Both the circadian rhythm in sleep propensity and the homeostatic pressure for sleep are modifiable by behavior and exogenous stimuli (Wyatt et al., 2004). One such stimulus, caffeine, is among the most widely used drugs in the world. Caffeine impacts sleep and wakefulness, to a large extent, by binding to adenosine receptors altering central and peripheral physiology (Porkka-Heiskanen and Kalinchuk, 2011). Many occupations require individuals to maintain long hours of wakefulness and work at night when the circadian clock promotes sleep. Therefore, caffeine is often used as a countermeasure to improve performance and reduce fatigue under such conditions of circadian misalignment (Roehrs and Roth, 2008; Wright et al., 1997a). Caffeine also increases CBT and attenuates its circadian decline (Wright et al., 1997b; Wright et al., 2000). One mechanism by which caffeine increases CBT is via vasoconstriction and decreased blood flow to the periphery (Pincomb et al., 1985; Hartley et al., 2004; Umemura et al., 2006), an action that may widen the DPG and influence sleep. Findings from studies investigating effects of caffeine administered prior to a daytime recovery sleep episode show decreases in stage 2 sleep, slow wave sleep (SWS; stages 3 and 4 combined), total sleep time (TST), and rapid eye movement (REM) sleep, along with increases in SOL, wakefulness after sleep onset (WASO), and the percent time spent in stage 1 sleep (LaJambe et al., 2005; Carrier et al., 2007; Carrier et al., 2009). This disturbed daytime recovery sleep architecture from caffeine is observed even though homeostatic pressure for sleep is high, as the circadian drive for wakefulness is also high (Czeisler and Gooley, 2007). In the latter caffeine studies, drug was administered 1h to 3h prior to the daytime recovery sleep episode. It is unknown whether caffeine taken approximately one

half-life prior to a daytime recovery sleep opportunity still disturbs sleep, and this was therefore examined in the current study.

It is also unknown whether caffeine influences the DPG, yet if it does, changes in skin temperature may represent a component of the integrative physiological response by which caffeine increases alertness and disturbs sleep. We hypothesized that caffeine administration during circadian misalignment and sleep deprivation, as common in night shift work, would widen the DPG, increase CBT, improve alertness and mood and disturb subsequent daytime recovery sleep. We also hypothesized that a greater widening of the DPG prior to sleep onset would be significantly correlated with a greater degree of sleep disturbance during daytime recovery sleep.

MATERIALS AND METHODS

Participants

Thirty healthy participants (n=9 females) aged 18-33 y (21.6±3.5 y; mean±SD), with BMI between 18.5-27 kg/m^2 (22.45±2.13 kg/m^2), and with self-reported habitual low to moderate caffeine use (>0mg and <500mg/day) participated. The control condition consisted of 20 healthy participants (5 female) aged 18-33 y (21.5 y±3.9 y), with BMI between 18.5-26.4 kg/m^2 (22.67±2.14 kg/m^2). The caffeine condition consisted of 10 healthy participants (4 female) aged 18-26 y (21.8 y±2.7 y) and BMI between 19.8-27 kg/m^2 (22.18±2.17 kg/m^2), values similar to the control group (p>0.55). Exclusion criteria included current smoker, drug use, pregnancy, habitual sleep duration <7h or >9h and any known medical, psychiatric or sleep disorder. Participants were deemed healthy based on physical exam, interview, psychological tests, blood chemistries (complete blood cell count and comprehensive metabolic panel), clinical

electrocardiogram, and medication free status. None of the participants reported regular night

work in the preceding year or crossing more than one time zone in the previous three weeks.

Health screening tests were conducted at the Clinical Translational Research Center (CTRC) of

the University Colorado Boulder. Participants were asked to maintain a self-selected sleep

schedule of ~8h per night for one week prior to admission to the laboratory, verified by sleep-

wakefulness logs, call-in bed and wake times to a time stamped voice-mailbox recorder, and

wrist activity with light exposure recordings (Actiwatch-L, Mini Mitter Respironics, Bend, OR).

Participants refrained from drugs (including caffeine and nicotine) for two weeks, and alcohol

two days prior to the laboratory session and throughout the protocol. Drug use was determined

by self-report and verified by urine toxicology at screening and by urine toxicology and a breath

alcohol tester (Lifeloc Technologies Model FC10, Wheat Ridge, CO) upon laboratory admission.

Participants gave written informed consent and the scientific advisory and review committee of

the Colorado Clinical and Translational Sciences Institute and the institutional review board

approved the research protocol.

Procedure

Participants lived in the Sleep and Chronobiology Laboratory at the University of

Colorado Boulder for ~3.7 days in a sound attenuated, temperature controlled, sleep and

chronobiology suite that was an environment free from time cues. Data presented in the current

report are from the first two days of a larger study for which portions have been published

(Burke et al., 2013). Participants were admitted to the laboratory ~4h prior to their habitual

bedtime. Females, six of which were free of oral contraceptives (four studied in follicular phase

and two in luteal phase) and three using oral contraceptives (two in pseudo-follicular phase and

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one in pseudo-luteal phase), were administered a pregnancy test to verify they were not pregnant. Polysomnography (PSG) was used to record an 8h baseline nighttime sleep episode at the participants' habitual bedtime. Following the baseline night, participants were tested using a 28h modified constant routine protocol. Room temperature was maintained in the thermoneutral range (22-24º Celsius) and lighting was maintained at dim ~1.9 lux (~0.6Watts/m^2) levels in the angle of gaze during scheduled wakefulness and 0 lux during scheduled sleep. During scheduled wakefulness of the constant routine, participants maintained a semi-recumbent seated posture in a hospital bed with the head raised to ~35 degrees, except for brief scheduled bathroom breaks (a commode <0.5m from the bed). After the first 16h of the constant routine, participants remained semi-recumbent and were provided urinals/bedpans when needed. During wakefulness, participants wore a light fitting tee-shirt and kept the bed sheet at waist level to maintain consistent microclimates of skin temperature recording sites. Participants received scheduled isocaloric meals every hour and completed a series of visual analog scales (VAS) every two hours until ~27h and 26h of scheduled wakefulness, respectively. Outside of the scheduled tests, participants had free time to engage in sedentary activities (e.g. read, watch movies, talk or play board games with a researcher, etc.). Wakefulness during constant routines was verified by continuous monitoring by research staff in the research suite and by continuous electroencephalography (EEG) recordings. Participants were informed they could receive pills that contained 5 mg melatonin, 2.9 mg/kg of caffeine or rice powder placebo. Participants who received melatonin did so later in the protocol after the timing of the data presented in the current analyses and thus, their data are included in the control condition. The CTRC pharmacist maintained double-blind conditions. Caffeine was administered double blind at 23h wakefulness

and at 28h wakefulness, participants were lowered to a supine position for a 5h daytime recovery

sleep episode in darkness (Figure 1).
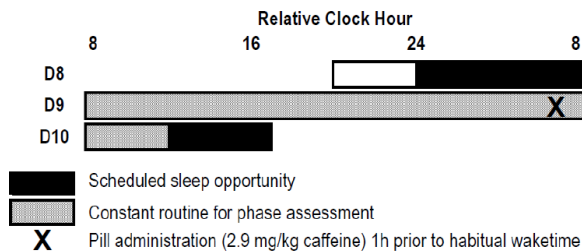
Figure 1. Diagram of the laboratory protocol. Procedures were scheduled according to each subject's habitual sleep and wake times. Grey shaded area indicates the modified constant routine protocol and black shaded areas indicates sleep opportunities. Caffeine administration is denoted with an X at 23h awake. The diagram shows an example protocol for a subject with a 08:00 habitual wake time. The first seven days of the study were comprised of subjects maintaining consistent wakefulness-sleep schedules at home and days 8-10 were in the laboratory.

Materials

Temperature Recordings

Skin temperatures were recorded every minute (iButton, Maxim, Sunnyvale, CA or Vital

Sense, Mini Mitter Respironics, Bend, OR) from the dorsal non-glabrous portion of the foot and

subclavian regions (van Marken Lichtenbelt et al., 2006). The DPG was calculated as the

difference between the distal foot (T_{Fd}) and the proximal subclavian region (T_{sub}). iButton

temperature sensors were taped to the skin surface using hypoallergenic surgical tape (Durapore,

3M, St. Paul, MN). Core temperature was sampled every minute with an ingested core

temperature capsule (Vital Sense, Mini Mitter Respironics, Bend, OR).

Polysomnography Recordings

PSG recordings were obtained with Siesta digital sleep recorders (Compumedics USA Ltd, Charlotte, NC) from C3-A2, C4-A1, O1-A2, F3-A2, right and left electrooculogram (EOG), chin electromyogram (EMG), and electrocardiogram (ECG). Sleep was manually scored in 30 second epochs according to standard guidelines from brain region C3-A2 (Rechtschaffen and Kales, 1968) or C4-A1 if C3-A2 contained artifact. Sleep onset latency was defined in two ways: 1) SOL, time from lights out to the onset of three continuous epochs of PSG defined sleep, and 2) the latency to persistent sleep (LPS), time from lights out to the onset of 10 continuous minutes of PSG defined sleep. We also calculated the latency to SWS (SWSL) and REM sleep (REML) from SOL.

Alertness and Mood Ratings

Participants' alertness and mood during sleep deprivation and following caffeine administration were assessed using VAS. Of the seventeen VAS items measured, we chose to analyze alertness, clear-headedness, relaxed, and sadness as we have found these items show high correlation with other VAS items during sleep deprivation (unpublished results).

Data Analysis

Skin temperature and CBT measurements were analyzed starting 2h prior to pill administration and continued throughout the 5h sleep episode. Individual T_{Fd} and T_{Sub} were averaged into 10 minute bins and DPG (T_{Fd} - T_{Sub}) was calculated. CBT was also averaged into 10 minute bins and analyzed as deviation from pre-pill baseline to control for individual differences in CBT level. CBT levels at baseline were 36.72^O C in the control and 36.61^O C in the caffeine condition (p=0.14). Effects of caffeine on DPG, CBT, $T_{Fd,}$ T_{Sub} and VAS were

analyzed using Mixed Model ANOVA with condition and time as fixed factors. Modified

Bonferroni correction factors were used to correct for multiple planned comparisons performed

using independent t-tests and to reduce type 1 error (Keppel, 1991). One subject in the caffeine

condition had missing data for the T_{Sub} and CBT and was therefore excluded from those analyses.

The first 5h of each sleep episode were analyzed using repeated measure ANOVA with

dependent or independent t-tests, as appropriate, for planned comparisons to examine the effects

of condition (control, caffeine), sleep episode (baseline, recovery sleep) and the interaction

between condition and sleep episode for SOL, LPS, percent and minutes of stage 1 sleep, stage 2

sleep, SWS, REM sleep, minutes WASO, SWSL, REML, and number and duration of

awakenings that lasted one or more epochs.

Pearson correlation coefficients were used to examine associations between changes in

the DPG and in the CBT (change in average temperature level for baseline 1h prior to the pill

administration to the average temperature level for the 1h immediately prior to the recovery sleep

episode) with percent stage 1, stage 2, SWS, REM sleep, and WASO, SOL and LPS for the

recovery sleep episode. Associations were also examined for average DPG and CBT levels

during the sleep episode and sleep measures. Correlations were first computed for the total

number of subjects irrespective of condition and then individually for each condition. Sleep and

temperature analyses were performed with STATISTICA, version 10.0 (StatSoft Inc, Tulsa,

OK), and correlation analyses were performed with Origin Pro 9 (OriginLab Corp, Northampton,

MA). Two subjects, one in the caffeine condition and one in the control condition, had missing

data for the $T_{Fd,}$ $T_{Sub,}$ and CBT for the hour immediately prior to the sleep episode and were

therefore excluded from the correlation analyses. During the sleep episode, two subjects in the

caffeine condition and one in the control condition had missing data for the DPG and the CBT.

One subject showed a sleep efficiency of less than 30% in the caffeine condition during the daytime recovery sleep episode and was identified as an extreme (Inter Quartile Range*3) outlier.   Therefore, the data from this subject was omitted from the analysis of sleep architecture during the recovery episode and correlation analyses. When this individual's data are included in the analyses, effects of caffeine on the reported sleep findings were even larger.

RESULTS

Effects of Caffeine on Alertness and Mood Ratings During Sleep Deprivation

Significant main effects of time for three of the four VAS items were observed with significantly lower alertness ($p<0.001$), less clear-headedness ($p<0.001$), and more sadness ($p<0.001$) across sleep deprivation (Figure 2A, B, C). A significant interaction of condition by time was also observed for alertness ($p<0.05$) (Figure 2A). Planned comparisons revealed higher clear-headedness and lower sadness 17h prior to caffeine administration (Figure 2B, 2C) and higher clear-headedness and alertness reported by participants in the caffeine condition 1h after caffeine administration (Figure 2A, B)

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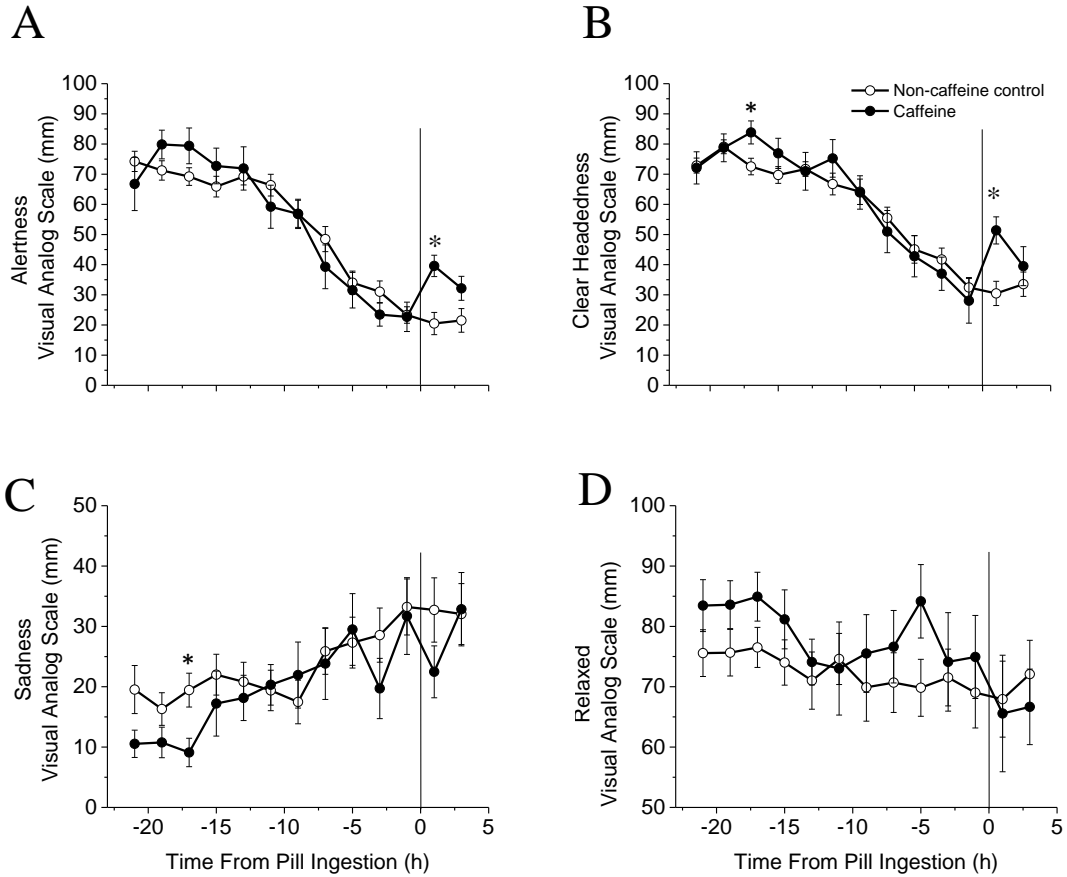
Figure 2. Mood and alertness during sleep deprivation. Visual Analog Scale (VAS) scores for the 2.9 mg/kg caffeine condition (closed circles, n=10) versus the non-caffeine control condition (open circles, n=20) during sleep deprivation prior to the daytime recovery sleep episode. The zero point represents the time of caffeine administration. A higher score on the y-axis denotes a higher rating for the VAS score plotted. * denotes a significant difference between conditions (p<0.05). Error bars are standard error of the mean. Note that the y-axis for sadness and relaxed are half of the range as those presented for alertness and clear headedness.

Effects of Caffeine on Body Temperature During Wakefulness

Skin and CBT levels were similar between conditions before caffeine administration (Figure 3). Caffeine significantly widened the DPG ($p<0.001$) and significantly increased CBT ($p<0.001$) (Figure 3A, C). There was a significant main effect of time ($p<0.001$) and interaction of condition by time ($p<0.01$) for both the DPG and CBT. Furthermore, there was a significant main effect of condition ($p<0.001$), time ($p<0.001$), and interaction of condition by time ($p<0.001$) for T_{Fd} (Figure 3B). There was also a significant main effect of condition ($p<0.001$) showing a higher T_{sub} after caffeine administration (Figure 3D). Planned comparisons revealed significant differences between caffeine and control in the T_{Fd} beginning 20 min after pill ingestion (Figure 3B), in the DPG beginning at 40 min after pill ingestion (Figure 3A), and in the CBT beginning at 60 min after pill ingestion (Figure 3C). There were no significant differences between caffeine and control conditions for the T_{sub} at individual time points.
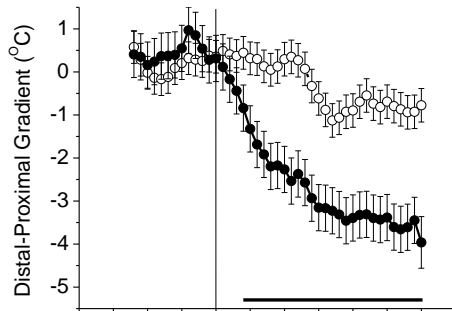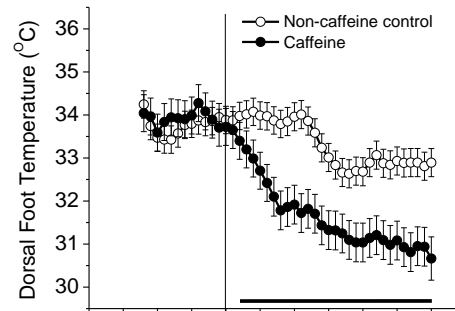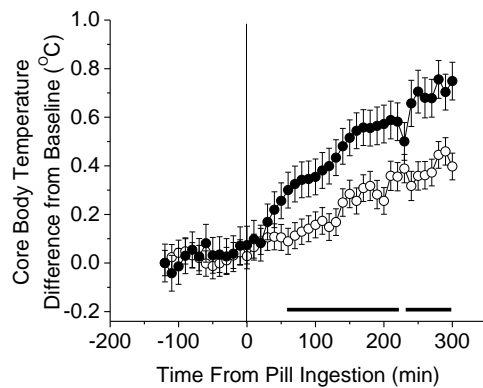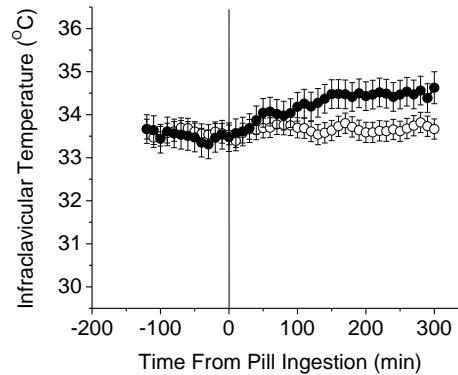
Figure 3. Body temperatures prior to daytime recovery sleep. Skin and core body temperature recordings for the 2.9 mg/kg caffeine condition (closed circles, n=9) versus the non-caffeine control condition (open circles, n=20) during sleep deprivation prior to the daytime recovery sleep episode. The zero point represents the time of caffeine administration. Bars under the data points near the x-axis denote significant differences between conditions (p<0.001). Error bars are standard error of the mean.

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Effects of Caffeine on Body Temperatures During Daytime Recovery Sleep

Caffeine significantly increased CBT and decreased the DPG during the sleep episode ($p<0.001$) and there was a significant main effect of time for CBT ($p<0.05$), and a non-significant trend for DPG ($p=0.06$) (Figure 4) showing a sleep induced decrease in CBT and sleep induced narrowing of DPG. Planned comparisons revealed significantly higher CBT for caffeine versus control from the start of the sleep episode to 190 min, between 210-220 min, and from 280 min to the end of the sleep episode ($p<0.05$; Figure 4 a), and significantly lower DPG in caffeine versus control from the start of the sleep episode to 40 min and between 60-70 min ($p<0.05$; Figure 4b) after lights out.
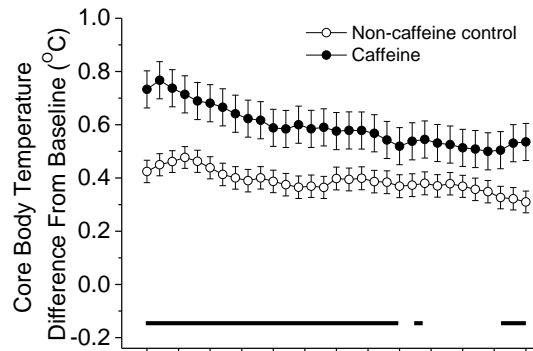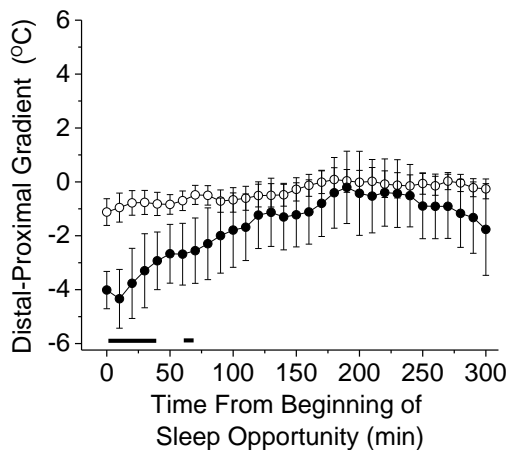
Figure 4. Core body temperature (CBT) and distal-to-proximal skin temperature gradient (DPG) during daytime recovery sleep. CBT and DPG recordings for the 2.9 mg/kg caffeine condition (closed circles, n=8) versus the non-caffeine control condition (open circles, n=19) after sleep deprivation during the daytime recovery sleep episode. The zero point represents the time of lights off and the beginning of the 5h sleep episode. Note data are a continuation of that shown in Figure 3C. Bars under the data points near the x-axis denote significant differences between conditions (p<0.048). Error bars are standard error of the mean.

Effects of Caffeine on Daytime Recovery Sleep: First 5h of Baseline versus 5h Recovery Sleep Episode Comparison

Table 1 shows sleep measures for baseline and daytime recovery sleep by condition. No significant differences in sleep measures were observed at baseline between conditions for the first 5h of recording (Table 1 and Table S1) or for the full 8h sleep episode (Table S2). Condition comparisons for the 5h daytime recovery sleep episode show that caffeine significantly reduced the percent SWS and SE, and the minutes of SWS and TST, with significantly increased WASO, SOL, LPS, and duration and number of awakenings compared to the control condition (Table 1). We also observed a non-significant trend for increased SWSL (p=0.08) in the caffeine condition compared to the control condition. In both conditions, daytime sleep consisted of significantly less percent and minutes of stage 2 sleep compared to baseline. Additionally, the control condition showed significantly higher percent and minutes SWS, higher percent SE, and lower percent and minutes of stage 1 sleep (Table 1) compared to baseline.

Table 1- Sleep architecture for the first 5h of each sleep episode

Measure	Control (n=20)			Caffeine (n=9)		
	Baseline	Recovery	P	Baseline	Recovery	P
Percent of 300 min Recording Time						
stage 1	4.4±0.5	3.7±0.5	0.0192	3.9±0.7	4.8±0.7	0.3179
stage 2	49.9±1.6	41.5±1.8	0.0000	52.0±2.4	41.1±2.7	0.0054
SWS	28.0±1.9	37.1±1.8	0.0000	24.3±2.9	28.1±2.7*	0.1646
REM	12.3±1.2	14.0±1.3	0.1394	13.6±1.9	16.1±1.9	0.3832
SE	94.6±0.7	96.3±1.2	0.0461	93.7±1.1	90.1±1.8*	0.2839
Minutes of Recording Time						
stage 1	13.1±1.5	11.1±1.4	0.0191	11.6±2.2	14.1±2.2	0.3837
stage 2	149.7±4.8	124.6±5.5	0.0000	155.9±7.2	118.6±8.1	0.0010
SWS	84.1±5.8	111.3±5.5	0.0000	72.8±8.6	81.3±8.2*	0.3939
REM	36.8±3.7	42.0±4.0	0.1400	40.8±5.6	47.4±5.9	0.4591
WASO	10.2±1.5	8.9±3.2	0.4818	10.9±2.2	23.6±4.7*	0.1840
TST	283.7±9.7	289.0±5.7	0.3428	281.1±8.9	261.3±7.2*	0.0219
SOL	6.1±1.2	2.1±0.6	0.0098	8.3±1.8	5.2±0.9*	0.0261
LPS	8.2±2.0	2.1±0.6	0.0077	13.4±3.0	6.6±0.9*	0.0425
SWSL	13.3±1.5	7.1±0.6	0.0000	15.3±2.2	8.9±0.8	0.0589
REML	112.8±11.4	62.7±2.6	0.0007	115.9±17.0	59.2±3.9	0.0028
Duration of Awakenings	0.9±0.1	0.9±0.1	0.9951	0.9±0.1	1.4±0.2*	0.1525
Number of Awakenings	10.6±0.9	10.2±1.0	0.7605	10.2±1.4	13.9±1.4*	0.1041

LPS = latency to persistent sleep; REM = rapid eye movement; REML = latency to REM sleep; SE = sleep efficiency; SOL= sleep onset latency; SWS = slow wave sleep; SWSL = latency to SWS; TST = total sleep time; WASO = wakefulness after sleep onset. p-values in table denote baseline versus recovery sleep comparisons within condition, *denotes p = <0.05 between conditions during the daytime recovery sleep episode, error bars are standard error of the mean.

Associations Between Changes in Temperature Prior to and during Sleep and Sleep

Architecture

Widening of the DPG prior to the daytime recovery sleep episode, including all subjects

in the analysis, was significantly associated with increased percent stage 1 sleep, SOL, LPS, and

minutes of WASO (Table S3, Figure 5A, B, C, D; $p < 0.05$) and was significantly associated with

decreased percent SWS and SE (Table S3, Figure 5E, F; $p < 0.05$). Widening of the DPG was not

statistically associated with percent stage 2 or REM sleep (Table S3). Similar findings were

observed for the DPG during the recovery sleep episode, except for SWS (Table S4). An

increase in the CBT prior to the daytime recovery sleep episode was significantly and negatively

correlated with LPS (Figure 6A) but not with any other sleep architecture measure. Non-

significant trends for a negative correlation with SOL (Figure 6B) and a positive correlation with

SWS were observed (Table S3). Average CBT during the sleep episode was not statistically

associated with any sleep architecture measure (Table S4)

Analyses for each condition separately show that for most sleep measures, associations

with temperature prior to sleep were in the same direction as that reported for correlation

analysis using the total of all subjects with some correlations being significant and some non-

significant trends (4 out of 6 DPG correlations for each condition; 2 of 2 correlations for CBT in

the control and 0 of 2 correlations in the caffeine condition that were significant or trends in the

total subject analysis; Figure 5). Sleep latency, however, showed opposite associations between

the change in DPG prior to sleep and SOL and LPS (Figure 5b,c) between conditions.

Specifically, the control condition showed longer latencies to sleep associated with narrower

DPGs prior to sleep, opposite to that for the caffeine condition and the total subject analyses

described above. Analyses for each condition separately for associations between temperature

during the sleep episode and sleep measures demonstrate condition differences showing

significant DPG findings only for the caffeine condition and significant CBT findings only for

the control condition (Table S4).
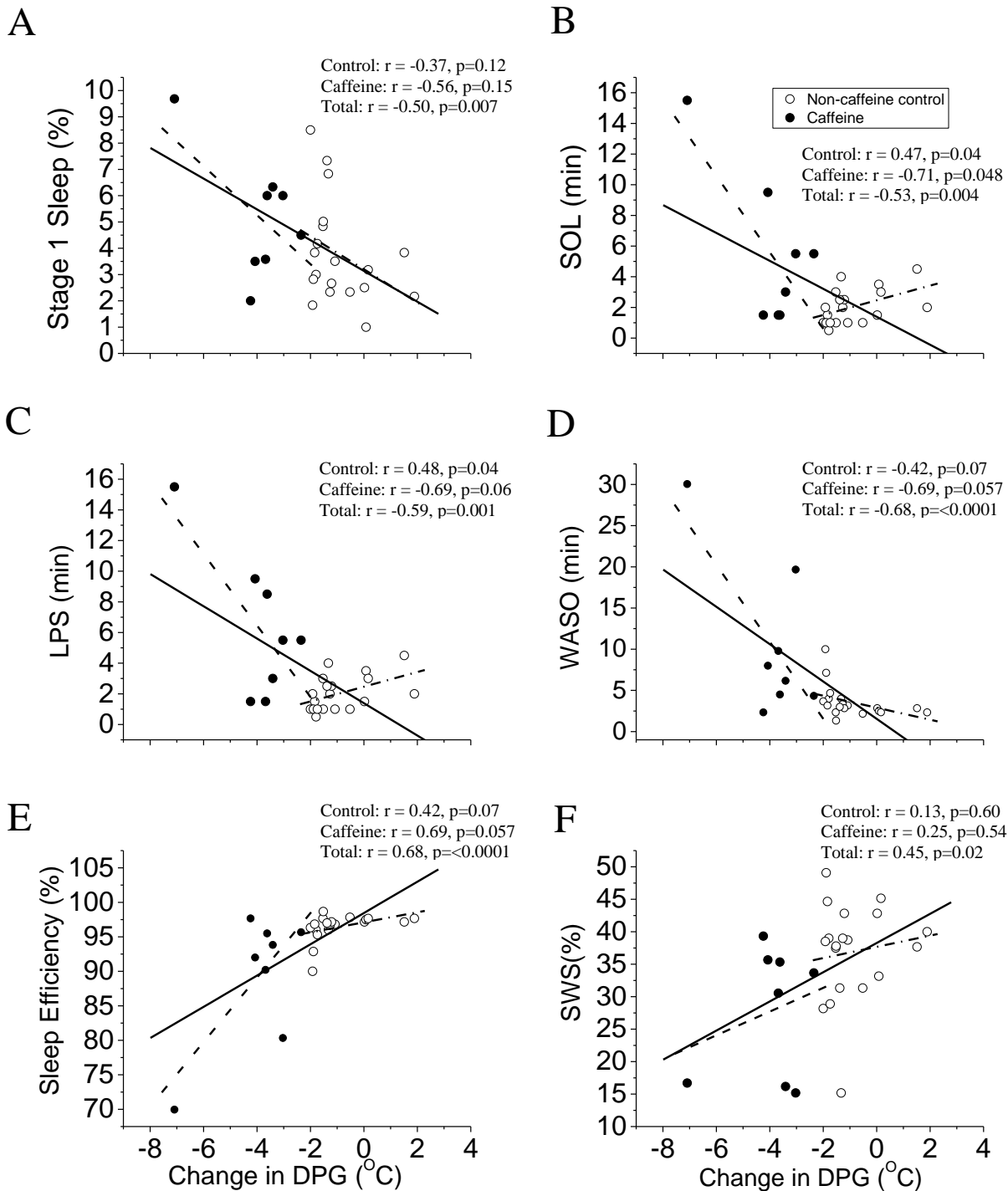
Figure 5. Associations between the change of the distal-to-proximal skin temperature gradient (DPG) and sleep. A more negative value of the DPG indicates a greater widening of the DPG prior to the daytime recovery sleep episode (i.e. the distal site is becoming less similar in temperature to the proximal site). The solid lines represent the best linear fit through both conditions combined (total subjects), the dashed lines represent the best linear fit through the control condition, and the dash-dot lines represent the best linear fit through the caffeine condition. Closed circles represent the 2.9 mg/kg caffeine condition (n=9) and open circles represent the non-caffeine control condition (n=19).

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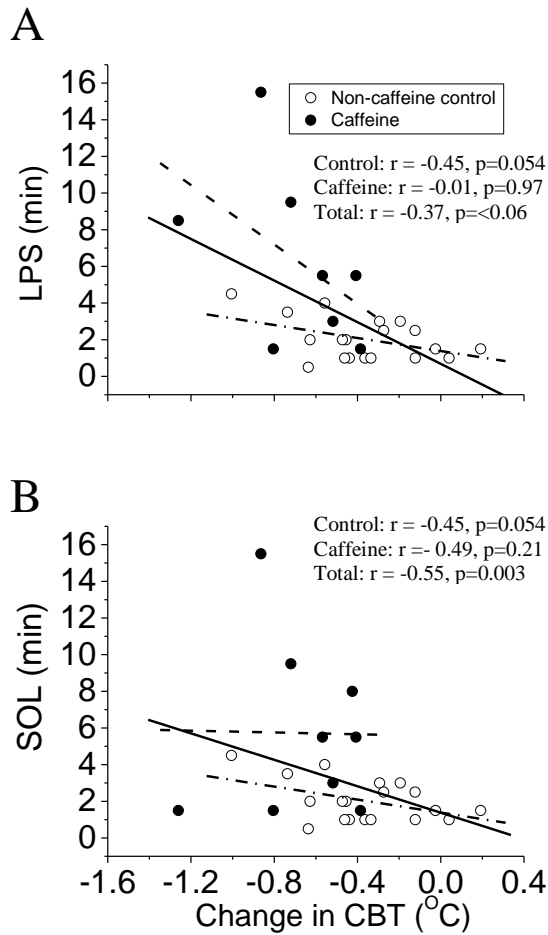
Figure 6. Associations between change in core body temperature (CBT) and sleep onset latencies. The solid lines represent the best linear fit through both conditions combined (total subjects), the dashed lines represent the best linear fit through the control condition, and the dash-dot lines represent the best linear fit through the caffeine condition. Closed circles represent the 2.9 mg/kg caffeine condition (n=9) and open circles represent the non-caffeine control condition (n=19).

DISCUSSION

Findings from the current study further our understanding of caffeine's influence on thermoregulatory physiology in the early morning hours during circadian misalignment, sleep deprivation and subsequent daytime recovery sleep as common in night shift work. We found that: 1) 2.9 mg/kg caffeine, equivalent to a double espresso, significantly widened the DPG primarily by attenuating heat loss through the periphery as observed via heat reduction in the foot 20 minutes prior to DPG widening and 40 minutes prior to CBT increases; 2) circadian misalignment and sleep deprivation reduced alertness and clear headedness and increased sadness, whereas caffeine administered at 23h awake acutely increased alertness and clear headedness 1h after administration; 3) caffeine disturbed daytime recovery sleep such that there was increased WASO, SOL, LPS, duration and number of awakenings and reduced SWS, TST and SE as compared to recovery sleep for the control condition; and 4) a widening in the DPG prior to the daytime sleep episode as well as during the sleep episode was associated with disturbed recovery sleep (i.e., longer SOL, LPS, more WASO, percent stage 1 and less percent SWS and SE) and an increase in CBT prior to the daytime sleep episode was associated with longer LPS.

Findings from the VAS indicate that self-reported alertness and mood were reduced by circadian misalignment and sleep deprivation (Dinges et al., 1997; Wright et al., 2002; Caldwell et al., 2004; Lieberman et al., 2005) and that caffeine administration increased alertness and mood, both consistent with prior findings (Lieberman et al., 1987; Penetar et al., 1993; Wright et al., 1997a).

Regardless of caffeine or control condition, circadian misalignment and sleep deprivation reduced LPS, REML, and minutes and percent stage 2 sleep, all reflective of higher homeostatic

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sleep pressure during daytime recovery sleep as compared to baseline sleep at night (Borbély, 1982; Borbély and Achermann, 1999; Carrier et al., 2007). The shorter REML during the daytime recovery sleep episode, regardless of condition, is consistent with the circadian rhythm in REM sleep propensity as the recovery sleep episode was scheduled just after the circadian peak for REM sleep (Dijk and Czeisler, 1995; Carrier et al., 2007).

As noted, in prior research studies on caffeine and daytime recovery sleep following sleep deprivation, caffeine was administered 1h to 3h prior to the daytime recovery sleep episode and was shown to increase SOL, SWSL, and the percent stage 1 sleep (LaJambe et al., 2005; Carrier et al., 2007; Carrier et al., 2009). Caffeine also decreased minutes of stage 2, SWS, REM sleep, TST and SE (Carrier et al., 2007). Our time of administration of caffeine 5h prior to recovery sleep is likely to be more common in real work operations than administration in closer proximity to sleep. As the half-life of caffeine is approximately 5-6 hours (Nehlig et al., 1992; Landolt et al., 1995; Keane and James, 2008), it might be expected that caffeine would have limited influence on sleep 5 hours later, although see (Drake et al., 2013). Our findings provide evidence that caffeine consumption ~1 half-life before sleep negatively affects daytime recovery sleep. Caffeine reduced the amount of SWS and as caffeine is an adenosine antagonist, this finding is consistent with a role of adenosine in the modulation of SWS (Schwierin et al., 1996). Caffeine, as compared to control, also increased WASO, SOL, LPS, duration and number of awakenings and decreased TST and SE as expected. Further research is needed to examine if the disturbed recovery sleep observed has consequence for next day cognitive and physiological functions.

Results from the correlation analyses for all subjects combined revealed that the more the DPG widened prior to sleep and during sleep there was greater sleep disruption as shown by

lower SE, longer SOL and LPS, more WASO, more percent stage 1 sleep and less percent SWS (prior to sleep only). When analyzing each condition separately, we found similar findings although some were no longer significant, perhaps related to the reduced sample size and variance in scores as the caffeine condition appeared to compress values towards the floor for some measures. Why SOL and LPS showed different associations with the DPG for the control and caffeine conditions is unknown and our control condition findings during daytime recovery sleep are inconsistent with DPG and SOL associations reported in the literature for nighttime sleep (Kräuchi et al., 1999; Kräuchi et al., 2000).

A growing body of evidence indicates that changes in skin temperature are associated with variations in alertness and sleep under bright light exposure (Cajochen et al., 2005), skin temperature manipulation via a thermal suit (Raymann et al., 2005; Raymann and Van Someren, 2007; Raymann et al., 2008), administration of melatonin or melatonin agonists (Kräuchi et al., 1997; Kräuchi et al., 2002; Aoki et al., 2008; Markwald et al., 2010), and now by administration of caffeine. Taken together, these findings provide evidence for a role of skin temperature changes in arousal states; although most findings reflect associations that require further examination at a mechanistic level using animal models.

Correlation analyses also revealed a positive association between the change in CBT prior to bedtime and LPS. The increase in CBT observed after caffeine administration is consistent with previous findings of higher CBT and longer latencies to sleep on the maintenance of wakefulness test after caffeine administration during nighttime sleep deprivation (Wright et al., 1997a; Wright et al., 1997b; Schweitzer et al., 2006). As the circadian CBT rhythm exhibits a rise in temperature in the morning prior to habitual waketime, the observation of increased CBT during wakefulness in both caffeine and control conditions was expected (Czeisler et al., 1980;

Zulley et al., 1981), as was the sleep induced decrease in CBT even though sleep occurred during the circadian rise of the CBT rhythm (Dijk and Czeisler, 1995). Yet, CBT remained higher after sleep onset for caffeine versus control for the majority of the sleep episode, and the DPG was initially wider after sleep onset for caffeine versus control.

Significant changes in distal skin temperature following caffeine were observed prior to changes in the DPG and core body temperature, indicating that the change in distal temperature represents the initial or a faster measured thermoregulatory response to a delayed caffeine response. Mechanisms by which the circadian increase in distal skin temperature may promote sleep and by which decreased distal skin temperature is associated with disturbed sleep and increased arousal are not fully understood. The preoptic anterior hypothalamus (POAH) is primarily responsible for thermoregulation (e.g. vasodilation/constriction of the vasculature, shivering and breathing rate) (Boulant and Dean, 1986) and POAH thermosensitive neurons have been found to affect sleep and wakefulness. Prior to sleep onset, warm sensitive neurons (WSN) in the POAH show increased firing rates and suppress arousal-related cell types whereas prior to wakefulness WSN show decreased firing rates (DeArmond and Fusco, 1971; Alam et al., 1995a; Alam et al., 1996). Local warming of the POAH thermoreceptor neurons has also been observed to induce sleep (Roberts and Robinson, 1969; Alam et al., 1995b). Likewise, warming of proximal and distal skin sites also increase POAH WSN firing rates (Boulant and Bignell, 1973; Boulant and Hardy, 1974; Van Someren, 2000). Therefore a narrowing of the DPG prior to sleep may trigger temperature sensitive neurons in the distal skin that excite WSN in sleep promoting areas of the brain to promote sleep or decrease arousal (Boulant and Bignell, 1973; Boulant and Hardy, 1974; Lowry et al., 2009). Conversely, caffeine administration and the observed widening of the DPG may decrease the excitement of the warm sensitive neurons, and after a

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delay disturb subsequent sleep and increase CBT. Furthermore, both adenosine receptors A_{1A} and A_{2A} are localized in most vascular beds in the periphery (Tabrizchi and Bedi, 2001), and acute adenosine administration can increase forearm blood flow by 572% (Smits et al., 1990). In contrast, acute caffeine administration constricts peripheral blood vessels leading to increased peripheral resistance and rises in blood pressure (Pincomb et al., 1985; Smits et al., 1990; Hartley et al., 2004; Umemura et al., 2006). Our findings suggest that caffeine, even when consumed 5h prior to sleep, may increase alertness and disturb subsequent daytime recovery sleep by binding to adenosine receptors in the brain and in the peripheral vasculature, both promoting arousal.

Centrally, adenosine receptors are present in several sleep-promoting regions of the brain (Porkka-Heiskanen and Kalinchuk, 2011; Brown et al., 2012). The wake-promoting basal forebrain is tonically inhibited by adenosine binding to its receptors causing the cell to hyperpolarize through increased potassium conductance (Basheer et al., 2004). This decreased neuronal activity is thought to result in increased sleep pressure that builds throughout wakefulness (Porkka-Hesikanen et al., 1997; Basheer et al., 2004). Adenosine antagonists, such as caffeine, increase the activity of wake-promoting neurons (Porkka-Heiskanen and Kalinchuk, 2011) and can subsequently disturb sleep.

The study contained several limitations. Temperature microclimates were present during the sleep opportunity as the subjects were permitted to sleep beneath blankets and this could have influenced both skin and core temperature measurements. Additionally, the findings of associations between the widening of the DPG and disturbed daytime recovery sleep do indicate causal relationships. Additional research is needed to explore dose and circadian time dependent administration effects of caffeine on thermoregulatory physiology, alertness and subsequent

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sleep during the daytime as well as at night, in different aged populations, low versus high

habitual caffeine users, in shift workers, and in patient populations such as those with insomnia

and shift work disorder.

In summary, the current findings indicate that the use of caffeine during circadian

misalignment and nighttime sleep deprivation alters thermoregulatory physiology, increases

alertness and clear-headedness, and disturbs subsequent recovery sleep when caffeine is

consumed 5h prior to the daytime sleep episode. Our findings demonstrate that a widening of the

DPG, primarily through decreased blood flow to the periphery, is associated with disturbed

daytime recovery sleep. Additional research is needed to provide evidence based

recommendations regarding the optimal dosage and timing of caffeine administration to

maximize alertness and minimize sleep disturbance during extended work operations that include

work at night and daytime recovery sleep.

ACKNOWLEDGMENTS

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CHAPTER 4

Supplementary Material

Effects of Caffeine on Skin and Core Temperatures, Alertness and Recovery Sleep During Circadian Misalignment

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Supplementary Table S1
Supplementary Table S2
Supplementary Table S3
Supplementary Table S4a and b

Table S1- ANOVA F-values for sleep measures during first 5h of each sleep episode

Measure	Condition	Recording Time	Condition x Recording Time
Percent of 300 min Recording Time	df=(1,27)	df=(1,27)	df=(1,27)
stage 1	0.15	0.16	5.27*
stage 2	0.09	53.09***	0.92
SWS	4.27*	24.15***	3.92
REM	0.82	2.98	0.10
SE	7.68**	0.56	5.00*
Minutes of Recording Time			
stage 1	0.09	0.05	4.46*
stage 2	0.00	71.88***	2.77
SWS	5.12*	16.81***	4.61*
REM	0.66	2.45	0.03
WASO	6.03*	3.19	4.85*
TST	10.89**	2.20	6.55*
SOL	3.83	10.20**	0.16
LPS	5.96*	12.95**	0.05
SWSL	1.48	26.74***	0.01
REML	0.00	27.08***	0.10
Duration of Awakenings	3.94	3.31	3.33
Number of Awakenings	1.66	2.38	3.49

*denotes p = <0.05, **denotes p= <0.01, *** denotes p=<0.001.

Table S2- Sleep architecture comparing 8h nighttime baseline sleep for each condition. No significant differences in sleep measures were observed between conditions at baseline when comparing the entire 8h sleep opportunity.

Measure	Control n=20	Caffeine n=9	t-test df (27)	P
	Baseline (480 min)	Baseline (480 min)		
Percent of Recording Time				
stage 1	5.0 ± 0.5	4.5 ±0.8	0.52	0.61
stage 2	50.0 ± 1.4	51.9±2.1	-0.75	0.46
SWS	19.9±1.3	16.0±2.0	1.6	0.11
REM	18.8±1.1	19.2±1.6	-0.21	0.83
SE	93.7±1.0	91.6±1.5	1.1	0.26
Minutes of Recording Time				
stage 1	23.9±2.4	21.7±3.6	0.51	0.61
stage 2	239.5±6.9	249.0±10.3	-0.77	0.45
SWS	95.5±6.5	76.7±9.6	1.6	0.12
REM	90.2±5.0	92.2±7.5	-0.23	0.82
WASO	23.9±4.6	32.1±6.9	-0.99	0.33
TST	449.1±4.9	439.6±7.3	1.1	0.29
Duration of Awakenings	1.5±0.4	1.8±0.6	-0.48	0.64
Number of Awakenings	18.2±1.3	18.8±1.9	-0.25	0.81

error bars are standard error of the mean.

Table S3- Associations between changes in temperature and daytime recovery sleep (n=27; total subjects available). Findings for DPG and CBT are described in the main text. Increased temperature of T_{FD} was significantly and positively correlated with percent stage 2 sleep, and SE, with a non-significant trend for SWS. T_{FD} was also significantly and negatively correlated with SOL, LPS, and WASO, with a non-significant trend for stage 1 sleep. Changes in T_{sub} were not associated with any sleep architecture measure, but showed a non-significant trend for negative association with SWS. By separating out the two locations used to determine the DPG, sleep disruption appears to be driven by decreased temperatures prior to sleep to the distal skin site as these associations mirror that of the DPG, while proximal changes were not associated with any sleep disruption.

Measure	Change in DPG prior to sleep episode		Change in Distal Foot (T_{FD})		Change in Proximal Subclavian (T_{sub})		Change in CBT prior to sleep episode	
Percent	Pearson r	P	Pearson r	p	Pearson r	p	Pearson r	P
stage 1	-0.50	0.007	-0.38	0.052	0.21	0.29	-0.19	0.35
stage 2	0.32	0.11	0.43	0.03	-0.19	0.33	-0.32	0.11
SWS	0.45	0.02	0.35	0.08	-0.34	0.08	0.33	0.09
REM	-0.24	0.24	-0.26	0.19	0.22	0.27	0.27	0.16
SE	0.68	<0.0001	0.70	<0.0001	-0.24	0.23	0.30	0.13
Minutes								
WASO	-0.68	<0.0001	-0.71	<0.0001	0.24	0.23	-0.30	0.13
SOL	-0.53	0.004	-0.57	0.002	0.14	0.47	-0.37	0.06
LPS	-0.59	0.001	-0.40	0.04	0.25	0.21	-0.55	0.003

Table S4a and b. Associations between average skin and core body temperature level and sleep measures during the daytime recovery sleep episode. Correlations were first computed for the total number of subjects available (S4a; n=27) irrespective of condition and then individually for the control (n=19) and caffeine (n=8) conditions (S4b). A narrower DPG during the sleep episode was significantly correlated with a higher percent SE and significantly correlated with lower percent stage 1, and minutes WASO, SOL, and LPS. Average CBT level was not associated with any sleep measure. These results are similar to the associations between the changes in DPG and CBT seen prior to sleep, with the exception of percent SWS with DPG and LPS with CBT, which are no longer significant for these analyses during the sleep episode. All significant associations observed for the DPG during sleep were in the same direction as those significant associations observed for the DPG prior to sleep. Individual condition correlations show associations that were less consistent during sleep.

Table S4a

Measure	Average DPG during the sleep episode (n=27)		Average CBT deviated from baseline during the sleep episode (n=27)	
Percent	**Pearson r**	**P**	**Pearson r**	**P**
stage 1	-0.39	0.0461	0.07	0.74
stage 2	0.22	0.27	-0.26	0.19
SWS	0.21	0.30	0.08	0.69
REM	0.21	0.30	0.25	0.21
SE	0.66	<0.001	0.06	0.78
Minutes				
WASO	-0.66	<0.001	-0.06	0.78
SOL	-0.62	<0.001	-0.13	0.51
LPS	-0.62	<0.001	-0.09	0.67

Table S4b

Measure	Average DPG during the sleep episode Control (n=19)		Average CBT during the sleep episode Control (n=19)		Average DPG during the sleep episode Caffeine (n=8)		Average CBT during the sleep episode Caffeine (n=8)	
Percent	**Pearson r**	**p**	**Pearson r**	**p**	**Pearson r**	**p**	**Pearson r**	**p**
stage 1	0.18	0.45	0.16	0.52	-0.81	0.01	-0.14	0.73
stage 2	0.16	0.51	-0.18	0.47	0.25	0.55	-0.45	0.26
SWS	-0.34	0.15	-0.12	0.63	0.46	0.25	0.56	0.15
REM	0.29	0.23	0.43	0.06	0.29	0.49	-0.09	0.83
SE	0.24	0.32	0.20	0.41	0.74	0.03	0.05	0.91
Minutes								
WASO	-0.24	0.32	-0.20	0.41	-0.74	0.03	-0.05	0.91
SOL	0.28	0.25	-0.54	0.02	-0.80	0.02	0.003	0.99
LPS	0.28	0.25	-0.54	0.02	-0.81	0.01	0.105	0.80

CHAPTER 5

CONCLUSIONS

Andrew W. McHill

Summary of Results

The purpose of this dissertation was to address the following deficiencies in our present

state of knowledge regarding how circadian misalignment influences energy metabolism,

cognitive performance, and thermoregulation in humans. First, it was unknown how eating

during the biological night, as common in overnight shiftwork, effects metabolic physiology and

increases risk for unwanted weight gain. Therefore, we tested the hypothesis that circadian

misalignment would lead to reduced fat utilization, a reduced TEF following food intake during

the biological night, and changes in circulating concentrations of appetitive and orexigenic

feeding hormones promoting increased appetite. We also hypothesized that circadian

misalignment would result in higher energy expenditure during the daytime sleep episode;

associated with greater number and duration of arousals during scheduled daytime sleep (Jung, et

al. 2011). We found that compared to baseline, total daily energy expenditure significantly

increased on the transition day to the first nightshift, which consisted of an afternoon nap and

extended wakefulness, and subsequently decreased on the second and third nightshifts, which

consisted of afternoon and nighttime wakefulness followed by daytime sleep, as compared to

baseline. Additionally we found that the thermic effect of food was reduced during the late night

dinner on the transition day as compared to baseline. Contrary to what was hypothesized,

sleeping energy expenditure was lower during daytime sleep than sleep at habitual time during

the night despite increased duration of arousals, total daily fat utilization increased on the first

two nightshift days, and ratings of hunger were decreased during night shiftwork despite

concurrent decreases in the satiety hormones leptin and peptide-YY.

Secondly, it was unclear how cognitive performance decrements during shiftwork change

across a first nightshift with extended wakefulness and subsequent overnight shifts as compared

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to a typical daytime working shift. We tested the hypotheses that cognitive performance would be worse on the first nightshift as compared to the baseline and subsequent nighttime working shifts, and that the performance during nighttime working shifts would be reduced as compared to the baseline daytime shift. We found that working during the night increased subjective sleepiness and decreased performance on the psychomotor vigilance task (i.e. decreased reaction time and increased attentional lapses), Stroop color word task (decreased number of correct responses) and mood. Further, sleepiness and mood increased from the first nightshift across subsequent nightshifts, but we observed limited evidence of performance changes across subsequent nights of simulated night work

Finally, it was unknown how caffeine influences thermoregulatory processes, particularly the distal-proximal skin temperature gradient (DPG), when consumed during sleep deprivation and circadian misalignment. Therefore we tested the hypothesis that caffeine during nighttime total sleep deprivation would reduce the DPG, increase CBT and alertness, and disturb subsequent daytime recovery sleep. We also hypothesized that a greater widening of the DPG prior to sleep would be associated with a greater degree of sleep disturbance. We found that prior to daytime recovery sleep, caffeine significantly widened the DPG, increased CBT, improved alertness and clear-headedness, and disturbed daytime recovery sleep. A wider DPG was associated with disturbed recovery sleep such that the wider the DPG, the increased amounts of WASO and stage 1 sleep and decreased amounts of sleep efficiency and SWS.

In summary, this dissertation aimed to improve our understanding of how circadian misalignment influences physiological mechanisms and increases risk for adverse health and performance outcomes. We showed that being awake and eating during the night and sleeping during the day decreases energy expenditure, and this could represent a contributing mechanisms

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by which eating at night increases the risk of weight gain and obesity. Furthermore, working during the night impaired cognitive performance on the first night of simulated shiftwork with extended wakefulness, and there was little evidence that these performance decrements changed across two subsequent night shifts. Lastly, caffeine consumed 5h prior to a daytime recovery sleep opportunity after sleep deprivation alters thermoregulatory physiology and represents a potential contributing mechanism by which caffeine disturbs sleep.

Future Directions

Our findings extend prior knowledge about the adverse health and cognitive outcomes associated with circadian misalignment. Possible extensions of this dissertation include the following. First, findings from previous literature have shown that eating during the night is a risk factor for weight gain (Arble, et al. 2009; Baron, et al. 2011). This dissertation demonstrated that wakefulness during the late afternoon and night and sleeping during the day decreased total daily energy expenditure by ~3% and eating a late dinner decreased the TEF as compared to eating during the daytime. These findings occurred despite controlling for caloric intake and activity. Future research is needed to examine total daily energy expenditure and energy balance when *ad libitum* food and activity are available during circadian misalignment. Further, this dissertation only studied healthy young (26.13± 4.5 y; mean±SD) individuals that were lean (BMI 22.67±1.7 kg/m^2) and had no history of disease. As shiftworkers are more likely to be overweight or obese and have comorbid disease (Bushnell, et al. 2010; Karlsson, et al. 2001), studying at risk populations such as older adults, overweight or obese individuals, or active shift workers is a necessary extension to be examined.

Second, although this dissertation demonstrated decrements in performance during the transition to the first night of shiftwork with extended wakefulness and on subsequent nights of overnight shiftwork, the decrements observed may not be directly translatable to real-work application. Incorporating tasks directly relevant to job specific duties is necessary to fully understand the degree of decrement, or if it is vulnerable to circadian misalignment. Additionally, the decrements in the dissertation were observed after daytime sleep opportunities in sleep promoting conditions (i.e. sound attenuated, complete darkness, temperature controlled room). Further examinations in more real-world settings that take into account family responsibilities, light exposure in the bedroom, daytime noise, temperature fluctuations, and prior drug use are necessary to understand the extent of performance decrements in nightshift workers.

Lastly, our administration of a single dose of caffeine 5h prior to daytime recovery sleep does not translate to all caffeine consumption during the nightshift. Additional research is needed to explore the circadian timing and dose dependent administration effects of caffeine on alertness, thermoregulation, and subsequent sleep during the daytime as well as at night.

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