IMPACT OF CIRCADIAN AND SLEEP DISRUPTION

ON METABOLIC HEALTH AND BEHAVIOR

by

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B.S., The Ohio State University, 2012

M.S., University of Colorado Boulder, 2015

A thesis submitted to the

Faculty of the Graduate School of the

University of Colorado in partial fulfillment

of the requirement for the degree of

Doctor of Philosophy

Department of Integrative Physiology,

Center for Neuroscience

2017

This thesis entitled:
Impact of Circadian and Sleep Disruption on Metabolic Health and Behavior
written by Ellen Rose Stothard
has been approved for the Department of Integrative Physiology
and the Center for Neuroscience

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The final copy of this thesis has been examined by the signatories, and we
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IRB protocol # 14-0018   12-0269   05-1072   16-0540

ABSTRACT

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Impact of Circadian and Sleep Disruption on Metabolic Health and Behavior

Thesis directed by Professor Kenneth P. Wright, Jr.

Wakefulness at the wrong biological time, referred to as circadian misalignment, as well as not obtaining enough sleep are primary risk factors for negative metabolic health outcomes, including diabetes, weight gain, and obesity. As much as 20% of the workforce in the US works non-traditional hours, some or all of which occur during the night. Additionally, an increasing portion of the population as a whole does not habitually obtain the recommended minimum of 7h of sleep per night.

Food intake is a critical component of energy homeostasis impacting weight gain and obesity. However, this is a complex behavior impacted by physiological and cognitive processes, which change under conditions of circadian misalignment and insufficient sleep. Here, we studied the effect of acute and chronic exposures to circadian misalignment with and without sleep disruption or food intake on physiology and cognition.

First, a simulated shift work protocol was used to examine the impact of acute circadian misalignment and insufficient sleep induced by simulated early morning shift work on metabolic outcomes. We found that food intake after waking under circadian misalignment induced elevated plasma glucose levels without a compensatory insulin response.

Secondly, simulated night shift work and total sleep deprivation protocols were employed to examine how hunger ratings change at night with and without food intake versus typical daytime conditions. We found that hunger ratings followed meal patterns and were lowest at night

regardless of food intake. Additionally, we found three factors that explained ~13% of the total variance in hunger rating changes: appetite for specific foods, overall hunger and desire to consume caffeine.

Finally, a protocol of chronic circadian misalignment was used to examine impacts on growth hormone (GH) secretion. During chronic circadian misalignment, GH secretion was decreased during sleep episodes that occurred during the biological daytime, whereas total 24h GH secretion remained unchanged.

Together, these findings demonstrate that metabolic hormones and hunger ratings are altered by acute and chronic circadian misalignment. Further studies are needed to continue to improve the understanding of how circadian misalignment impacts cognitive function and physiological processes contributing to metabolic dysregulation.

DEDICATION AND ACKOWLEDGMENTS

This dissertation is dedicated to all the wonderful people in my life who have supported and encouraged me. Especially, I would like to dedicate this dissertation to my family: my mother for bringing me into the world and teaching me the importance of quality time, my father for always reminding me that doing something you love is never work, and my sister for never letting me take things too seriously. As well as to all my friends who have been like family, by my side on some of my greatest adventures and without whom I would not be where I am now.

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CHAPTER 1

EFFECTS OF CIRCADIAN DISRUPTION AND INSUFFICIENT SLEEP

ON ENERGY HOMEOSTASIS

Ellen R. Stothard

Introduction

Integration of behaviors and biological processes throughout the body is essential for optimizing systemic physiological function. Healthy functioning allows for appropriate behaviors to occur at optimal times, including coordinating tissues, hormones, and behavior such that wakefulness and activity occur at appropriate times. Physiology has evolved to integrate exogenous and endogenous signals to maintain this timing rhythm for optimal function and survival. Demands and developments of modern society have changed the relationship between the external and internal environments, potentially threatening this coordination. The invention of the electrical light has allowed for wakefulness to be extended far into the solar night, thus creating an artificially lengthened solar day and allowing for the expansion of social engagements and work responsibilities much later than ever before.

These changes in 24-hour demands of modern society have recently been implicated in increases in chronic disease prevalence with more than 2.1 billion adults worldwide diagnosed with either overweight or obesity (Finucane et al, 2011). Between 1980 and 2013, global rates of obesity increased by 28% in adults and 47% in children (Ng et al, 2014). Data from the National Health and Nutrition Examination Surveys (NHANES) estimates that obesity effects 35% of adults in the United States (Fryar et al, 2012) and as much as half of all adults in some countries (Ng et al, 2014). Overweight and obesity significantly increase risk of comorbid cardio-metabolic conditions, such as cardiovascular disease (Klein et al, 2004), hyperlipidemia (Eckel, 2001), diabetes (Eckel, 2001; Patterson et al, 2004), and sleep apnea (Tung, 2005).

Concurrent with the increase in overweight and obesity, a decline has been reported in the number of people obtaining adequate sleep (Knutson and Van Cauter, 2008) with an estimated one-third of the United States population sleeping less than the recommended seven

hours per night (Watson et al, 2015; Liu et al, 2016). This sleep disturbance could be insufficient sleep duration, irregular timing of sleep episodes, poor quality sleep, or some combination resulting in insufficient sleep. Many proposed mechanisms connecting sleep and metabolic function. Insufficient sleep has been associated with diabetes (Aurora and Punjabi, 2013), cardiovascular disease (Puttonen et al, 2012), the metabolic syndrome (Van Cauter et al, 1997b; Bass and Turek, 2005; Chaput et al, 2013; Depner et al, 2014), mood disorders (John et al, 2005), as well as weight gain and obesity (Spiegel et al, 1999; Taheri et al, 2004; Gangwisch et al, 2005; Knutson and Van Cauter, 2008). Insufficient sleep can occur independently or can also be present concurrently with required wakefulness and activity at abnormal biological times. More than 20% of the population has been shown to work non-traditional or variable hours outside the "typical" 9:00 AM to 5:00 PM work day, as in shift work (McMenamin, 2007). Being awake during the biological night desynchronizes biological, or circadian, timing and normal sleep and wakefulness behaviors, which in turn disturbs normal physiology and leads to a state of circadian misalignment. Circadian misalignment is common in shift work when wakefulness and associated activities such as cognitive demands, physical activity, and food intake occur during the biological night and sleep and related activities, including fasting and inactivity, occur during the biological day, increasing the risk of disturbed sleep. The interconnectedness of the causes and risk factors for obesity, sleep restriction and circadian misalignment demonstrates the difficulty as well as the importance of the study of these systems.

Multiple factors are involved in the pathogenesis of obesity, this comprehensive review will explore the current state of knowledge, providing an overview of the circadian system, sleep and wakefulness and research techniques. Additionally, a review of energy metabolism including energy balance and weight gain will be provided with discussion of perturbations in normal

circadian and sleep physiology and the resulting impact on energy metabolism, and related physiology and behavior. Finally, connections to endocrine hormone growth hormone will be explored.

The Circadian System

Human survival and activity requires the coordination of a variety of physiological functions from autonomic processes to energy intake to decision making as well as recovery via sleep. Under optimal conditions these and many more physiological process are coordinated. For example, wakefulness, cognition and energy metabolism are promoted during the biological day and sleep and restorative processes during the biological night. A central pacemaker is required to keep time internally and maintain proper timing of physiological processes.

Components and Function of the Circadian System

The suprachiasmatic nucleus (SCN) was identified as the central circadian pacemaker in mammals which sends direct and indirect signals throughout the body, endogenously coordinating daily behavioral and physiological patterns both internally and with the environment (Winfree, 1967; Moore and Eichler, 1972; Stephan and Zucker, 1972; Wever, 1979; Kronauer et al, 1982; Pickard and Turek, 1983; Kronauer and Czeisler, 1993; Sakurada et al, 2000; Ruiter et al, 2003; Scheer et al, 2003; Saper et al, 2005; Scheer et al, 2005; Saper et al, 2010). The SCN is a bilateral structure which contains approximately 50,000 neurons in humans and 20,000 neurons in rats (Guldner, 1983; Swaab et al, 1985; Hofman et al, 1988; Klein, 1991). The SCN is located in the anterior hypothalamus, sits above the optic chiasm and next to the third ventricle. Cellular timekeeping within the SCN neurons is carried out by a

transcriptional translational feedback loop. Activators CLOCK (circadian locomotor output cycles kaput) and BMAL1 (brain and muscle Arnt-like protein 1) dimerize in the cytoplasm, translocate to the nucleus and bind promoter regions on the *Period (Per)* and *Cryptochrome (Cry)* genes, activating transcription (King et al, 1997; Gekakis et al, 1998; Hogenesch et al, 1998; Reppert and Weaver, 2002). Levels of PER and CRY proteins subsequently increase, activating the negative feedback arm of this cycle. PER/CRY heterodimers translocate back to the nucleus and inhibit CLOCK/BMAL, which results in decreased transcription of *Per* and *Cry* genes (Zylka et al, 1998; Jin et al, 1999; Kume et al, 1999; Okamura et al, 1999; Vitaterna et al, 1999; Shearman et al, 2000; Reppert and Weaver, 2001; Preitner et al, 2002; Yu et al, 2002). PER/CRY heterodimers are degraded over time releasing the repression of *Per* and *Cry* gene transcription and oscillating with a cycle length, or period, of 24 hours demonstrated *in vivo* by increased neuronal firing during the biological day and decreased firing during the biological night (Gillette and Reppert, 1987). *In vitro,* SCN slices from rats exhibited a rhythm in culture similar to the light/dark cycle of the donor or control rats (Green and Gillette, 1982; Groos and Hendriks, 1982). Transplanting the SCN in rodents imparts the period of the donor into the recipient (Ralph et al, 1990). In humans, indirect measures of biological timing show individual differences are genetically-determined, with an average period of 24.15 hours (Czeisler et al, 1999; Wyatt et al, 1999; Wright et al, 2001; Wright et al, 2006; Gronfier et al, 2007; Duffy et al, 2011). Differences from precise 24 hour rhythmicity demonstrate the need for external inputs into the SCN to prevent drifting and maintain synchrony with the external environment for optimal function.

Circadian Photoreception Maintains Synchrony with the External Environment

Many external factors influence central or peripheral circadian timing including food intake (Damiola et al, 2000; Stokkan et al, 2001), exercise (Klerman et al, 1998), and caffeine (Burke et al, 2015). However, light has been demonstrated as the primary synchronizing input or zeitgeber (German for "time giver") for the central circadian clock in the SCN (Rusak and Zucker, 1979; Czeisler et al, 1986; Czeisler, 1995; Shanahan et al, 1997; Boivin and Czeisler, 1998; Czeisler CA, 1999; Shanahan and Czeisler, 2000; Zeitzer et al, 2000; Wright et al, 2001; Gronfier et al, 2004; Scheer et al, 2005; Gronfier et al, 2007). Synchronization or entrainment, to the external environment is important for survival. Light is sensed by retinal photoreceptors and this photic information is transmitted to the SCN via the monosynaptic retinal-hypothalamic pathway (RHT) (Foster et al, 1991; Morin, 1994; Argamaso et al, 1995; Moore et al, 1995; Lucas et al, 2001; Wright and Czeisler, 2002). The RHT originates from a subset of retinal ganglion cells which are intrinsically photosensitive (ipRGCs) and contain the photopigment melanopsin and have been shown to impact timing of the central clock (Moore, 1995; Brainard et al, 2001; Hattar et al, 2002; Provencio et al, 2002; Lockley et al, 2003). Rods and cones also transmit information to the SCN via ipRGCs (Guler et al, 2008; Lall et al, 2010). Under conditions of constant darkness, findings from animal models have demonstrated that the circadian clock continues to oscillate with its endogenous period, or "free run"(Pittendrigh and Daan, 1976; Meijer and Rietveld, 1989). Thus, as light levels in the environment change, that information is sent to the SCN to coordinate optimal functioning based on that specific time of day.

Markers of the Circadian System

Timing of the human circadian system can be assessed through a number of marker rhythms controlled by the SCN. Core body temperature displays a circadian rhythm with peak body temperature occurring during the day and the trough occurring at night (Duffy et al, 1998; Dijk et al, 1999). Cortisol levels also exhibit circadian variation with a peak just before habitual wake time (Bradbury et al, 1991) and a trough at night (Dorn et al, 2007). Secretion of the pineal hormone melatonin is tightly controlled by the SCN, making it a reliable marker of circadian timing. Melatonin is measurable in the blood and saliva and its metabolites are present in urine, allowing for ease of assessment under both research and field conditions (Deacon and Arendt, 1994). Assessment of multiple time points can provide comparison for relative circadian time, but sampling 24 hour melatonin levels is considered the most robust phase marker of the mammalian circadian clock (Van Someren and Riemersma-Van Der Lek, 2007). Multiple days to weeks of sampling the 24h melatonin, core body temperature, or cortisol rhythm also assess circadian period. Melatonin is secreted in low concentrations throughout the biological day, increasing approximately two hours prior to bed time (Dim Light Melatonin Onset or DLMO), is elevated during the biological night and then decreasing to mark the end of the biological night (Dim Light Melatonin Offset or DLMOff) (Lewy and Sack, 1989; Lewy et al, 1999). Rodents conserve this same pattern of melatonin secretion even though, in contrast to humans, their natural active period occurs during the dark phase when melatonin is high and their natural inactive period occurs during the light phase when melatonin is low (Armstrong, 1989). Melatonin is synthesized in a multi-step process, beginning with tryptophan converted to serotonin by tryptophan hydroxylase (Klein et al, 1981). Melatonin is then synthesized from serotonin by N-acetylation (by N-acetyltransferase, NAT) and subsequent O-methylation by

hydroxyindole-O-methyl transferase (HIOMT) (Axelrod and Weissbach, 1960; Weissbach et al, 1960). NAT activity is increased during the biological night and is thought to be the cause of increased melatonin synthesis (Ebadi, 1984). Melatonin is produced by the pineal gland and then released into circulation to interact with melatonin receptors on diverse types of tissues, regulating biological functions including locomotor activity, reproduction, body temperature and feeding (Arendt et al, 1995; Zawilska and Nowak, 1999; Goldman, 2001; Pevet, 2002). Melatonin also has sleep-promoting effects (Dijk and Cajochen, 1997; Sack et al, 1997; Zhdanova et al, 1997) and exogenous melatonin administration in humans increases total sleep time in daytime sleep episodes (Dijk et al, 1997; Wyatt et al, 1999; Wyatt et al, 2006). Light exposure at night has been shown to suppress melatonin secretion (Lewy et al, 1980; Zeitzer et al, 2000; Duffy and Wright, 2005) and blue light has shown greatest suppression effects at normal light intensities (~150 lux) (Brainard et al, 2001; Gooley et al, 2010). Assessment of circadian timing provides insight into physiological functioning and the widespread impact of disrupting these systems.

Sleep and Wakefulness

Sleep and wakefulness are behavioral states associated with specific physiological functions and brain activity. Sufficient sleep is important for maintenance of normal functioning and sleep loss has been associated with many negative health outcomes including death in animal models exposed to chronic total sleep deprivation (Rechtschaffen et al, 1983). Sleep is important in that its functions support and promote the health of many other physiological systems.

Physiological Characteristics and Neuronal Substrates of Sleep and Arousal

Sleep is identified by a species-specific posture and reduced responsivity to external stimuli (Zeppelin, 1994). Sleep is distinguished from states such as hibernation by the quick reversibility of conscious awareness. In mammals, sleep is also accompanied by specific patterns of electrical activity in the brain. Electroencephalography (EEG) is a technique that uses electrodes placed on the scalp in humans or directly in specific brain areas in animals to assess sleep and wakefulness states. Wakefulness is a behavioral state characterized by desynchronized, high-frequency cortical EEG in the 14-30 Hertz (Hz) or cycles per second, range. The ascending arousal system that controls wakefulness consists of two branches (Saper et al, 2005). The first branch originates in the pons with the acetylcoline-producing (ACh) cell groups, the pedunculopontine nucleus (PPT) and the lateral dorsal tegmentum (LDT) and facilitates thalamocortical communication. The second pathway, activates the cortex, lateral hypothalamic area and cholinergic basal forebrain through projections from the brainstem and hypothalamus including the noradrenaline-containing locus coeruleus (LC) neurons, serotonin-containing dorsal raphe nucleus (DR) neurons, dopamine-containing ventral lateral periaqueductal grey matter (vlPAG) neurons and histamine-containing tuberomammillary neurons (TMN)(Saper, 1985; Saper et al, 2001; Jones, 2003). The ventrolateral preoptic nucleus (VLPO) projects to the monoaminergic areas involved in arousal including the TMN, LC, PPT, LDT, and lateral hypothalamus (LH). VLPO neurons contain inhibitory neurotransmitters galanin and GABA (Sherin et al, 1998; Gaus et al, 2002) and are active during sleep, but can also be inactivated by projections from the monoaminergic areas including LC, DR, TMN (Vincent et al, 1982; Gallopin et al, 2000; Chamberlin et al, 2003). Specific projections from the VLPO control the balance of and transitions between different stages of sleep. The VLPO output to the LC and DR

help initiate rapid eye movement (REM) sleep (Lu et al, 2002; Verret et al, 2005) and to the

TMN are involved in the transition between wakefulness and non-REM sleep (Lu et al, 2002;

Ko et al, 2003; John et al, 2004). Orexin (also known as hypocretin) is produced by the LH and

has projections to arousal centers to reinforce stability of wakefulness state (Chou et al, 2002;

Sakurai et al, 2005; Yoshida et al, 2006).

Sleep is Characterized in Discrete Stages

This mutually inhibitory ability of VLPO neurons in this circuit creates a "flip-flop

switch" which characterizes sleep in discrete states with few transitional states. Rechtschaffen

and Kales (Rechtschaffen A, 1968) created the manual by which sleep stages are identified using

continuous EEG measurement. Prior to the onset of sleep, the brain transitions into a state of

"quiet wake" which is characterized by alpha waves in the 8-12 Hz range. Transition to each

subsequent stage is considered to increase in sleep depth. The next stage of sleep is non-rapid

eye movement (NREM) Stage 1. NREM stage 1 is characterized by decreased conscious

awareness, slowing or decreased frequency of EEG, the appearance of theta waves in the 4-7 Hz

range, and is the lightest stage of NREM. The next stage is NREM Stage 2, characterized by

complete loss of conscious awareness, and the appearance of sleep spindles and K-complexes.

The final two stages of NREM sleep are stages 3 and 4, which are often grouped together and

referred to as "deep slow wave sleep". They are characterized by the appearance of delta waves

in the 1-3 Hz range, which is also called slow wave activity (Achermann and Borbely, 2003).

Increasing sleep intensity is defined as an increase in total power, amplitude and appearance of

delta waves in NREM sleep. During a normal night of sleep the brain cycles through increasing

sleep intensity and then back up to lighter stages of sleep before initiating the first episode of

rapid eye movement (REM) sleep in the first 80 to 100 minutes of the sleep episode. REM sleep

is characterized by high frequency, low amplitude sleep similar to NREM 1 or waking EEG in

rodents and non-humans. In humans, REM sleep resembles Stage 1 theta waves in the 4-7 Hz

range. During REM sleep all skeletal muscles experience atonia, only inner ear, respiratory,

ocular, and smooth muscles are unaffected.

Sleep Duration and Timing

Throughout sleep, the brain will cycle through increasing intensity of NREM sleep

stages, into REM and back into lighter stages of sleep approximately every 90 minutes across the

night. REM sleep episodes increase in length through the night and are present in greater

duration in the second half of the night, whereas slow wave sleep (SWS) appears oppositely and

is present in greater duration in the first half of the night. Stage 2 is present equally throughout

the night and alternates with REM sleep for most of the second half of the night. The pressure for

REM sleep in the latter half of the night is modulated by the circadian system (Czeisler et al,

1980; Zulley, 1980), whereas SWS reflects build-up of pressure from the length of previous

wakefulness, or homeostatic drive for sleep (Weitzman et al, 1980; Dijk and Czeisler, 1995).

Thus the timing and duration of sleep has an impact on the architecture of sleep, as the timing

and duration of sleep restriction also affects sleep architecture. Sleeping at an adverse circadian

time results in the greatest amount wakefulness after sleep onset (WASO) during the sleep

episode, which is a marker of fragmented and poor quality sleep (Dijk et al, 1997; Wyatt et al,

1999). Disrupted sleep decreases the quality and effectiveness of the sleep episode which can

have functional consequences during the subsequent wake episode. Proper timing and duration

of sleep opportunity is important to maintain the quality and effectiveness of sleep's physiological function.

The Two Process Model of Sleep Homeostasis and Circadian Regulation

The integration of the circadian and sleep systems and processes involved in the regulation of sleep and wakefulness is described as a Two-Process Model (Borbely, 1982). This model attempts to characterize observed daily variation in alertness, performance and physiological function as a result of an interaction between two processes: sleep (Process S) and circadian (Process C). Process S is a homeostatic drive for sleep, which builds across wakefulness in direct proportion with the length of time awake. During sleep this homeostatic drive is dissipated. Process C is defined as the circadian drive for wakefulness. The strength of Process C varies across the day to counteract the homeostatic drive for sleep. Process C is low in the morning when the homeostatic drive for sleep is low, and increases slowly across the day. In the late afternoon, a decline in performance is seen because the circadian drive for wakefulness is not sufficient to counteract the homeostatic drive for sleep. Near the beginning of the biological night Process C decreases, allowing initiation of sleep, and then remains low throughout the night resulting in consolidated sleep. As previously discussed, the circadian system oscillates independently to maintain synchronization of wakefulness and sleep to the light-dark cycle (Borbely, 1982; Czeisler et al, 1990; Dijk et al, 1992; Johnson et al, 1992; Dijk and Czeisler, 1995; Cajochen et al, 1999; Wyatt et al, 1999; Achermann and Borbely, 2003; Durmer and Dinges, 2005). In the event of sleep loss, the circadian system will continue to oscillate as normal. This is a concern for overnight and shift workers as their biological clock is not

promoting wakefulness to counteract the homeostatic drive for sleep during their biological night when they are on the job.

Circadian and Sleep Research Protocols

As the circadian system and sleep physiology are highly integrated, study of their discrete impacts on physiology has historically proven complicated. Therefore, specific protocols have been designed to systematically manipulate circadian and sleep variables. Important protocols in the circadian and sleep research fields are the constant routine, the forced desynchrony, and simulated shift work. The constant routine protocol controls the influence of behavioral and environmental factors through constant wakefulness, limited movement, controlled posture, evenly distributed isocaloric food intake, constant ambient temperature, and dim light exposure (Mills et al, 1978; Czeisler et al, 1986; Krauchi and Wirz-Justice, 1994; Duffy and Dijk, 2002). Controlling or distributing environmental and behavioral influences across the circadian cycle and removing sleep allows this protocol to help determine the effects of the circadian system on variables. A limitation of this method is that circadian influence cannot be determined by the results of this protocol alone, as there may be concurrent impacts of sleep loss on the variable of interest. Also, for health and safety reasons the protocol often is only able to study the acute impacts of sleep loss, leaving the possibility that chronic exposure or multiple circadian cycles would demonstrate different results. In addition, the results may not be translatable to normal function, as constant conditions are not common and total sleep deprivation is difficult to endure. Another circadian research protocol, the forced desynchrony, evenly distributes behavioral influences (sleep, food intake, wakefulness, etc) across the circadian cycle allowing for the mathematical separation of the two variables (Kleitman, 1963; Dijk and Czeisler, 1995; Dijk et

al, 1997; Czeisler et al, 1999; Cajochen et al, 2002). The protocol uses dim light and extends or shortens the length of a "day" outside the body's ability to synchronize, or entrain, its circadian timing causing the circadian system to "free-run" or continue to oscillate at its own rhythm. Standard day lengths are 20 and 28 hours, though other lengths are used with sleep and wakefulness taking place at a 1:2 ratio to mimic normal behavior. The protocol takes place over multiple cycles of these "days" allowing for sleep and wakefulness episodes to occur at all different circadian times. Physiological function and other research outcomes can then be studied under identical sleep and wakefulness conditions at varying times of the circadian clock. Limitations of the forced desynchrony protocol include inability to separate the impacts of sleep independent of the circadian fluctuations and high cost and time burdens as these studies take place over multiple days, weeks and even months. A final circadian and sleep research technique is a shift-work simulation (Eastman and Martin, 1999; Sharkey et al, 2001; Lamond et al, 2003; McHill et al, 2014). In a simulated shift-work protocol, wakefulness and activity is scheduled to occur overnight and sleep is shifted to the daytime. This allows for the assessment of circadian influence on wakefulness, activity and sleep. Feeding and other variables of interest can also occur overnight assessing the influence of circadian misalignment. A limitation of this protocol is that daytime sleep is often fragmented which may cause it to be insufficient and this could additionally impact the research variables. These research protocols are standard methods by which researchers manipulate sleep and circadian function to understand mechanisms of and changes in physiological functioning. Understanding the methods and limitations of these protocols can assist in the interpretation of results of these studies, providing insight into not only normal physiological functioning but the results of disturbances in function.

Components of Energy and Metabolic Processes

Continuous function of all biological systems requires that energy be consumed, stored and available for use, which is accomplished through the synergistic balance and functioning of many metabolic processes. Balancing energy intake and use can promote weight maintenance and help prevent weight gain and obesity.

Components of Energy Balance

The energetic cost of daily functioning including ambulation, metabolic processes, and sleep, among others, is termed total daily energy expenditure (EE). Specifically, total daily energy expenditure consists of the thermic effect of food, resting metabolic rate, and activity energy expenditure (Donahoo et al, 2004). First, the thermic effect of food is the increase in metabolic rate after ingestion of a meal due to energy required to digest, absorb, utilize and store food (Reed and Hill, 1996). The resting metabolic rate is the energy expended by physiologic processes under wakeful conditions (Haugen et al, 2003). The final components of total daily energy expenditure are activity energy expenditure, the energy required to complete exercise related processes, and non-exercise energy expenditure, such as fidgeting or other movements not considered exercise. Sleeping metabolic rate is also important to the calculation of total daily energy expenditure as it is lower than the resting but wakeful metabolic rate (Jung et al, 2011). Overall, taking in and expending an equal amount of energy results in weight maintenance, while excess energy intake leads to positive energy balance resulting in weight gain, whereas, negative energy balance leads to weight loss. An important aspect of the research examining how energy balance is maintained involves dissecting the components involved in the energy balance equation and studying how they interact. Not only are there many aspects to energy expenditure,

as explained previously, energy intake is a multifaceted process that involves more than total caloric intake. Maintenance of energy homeostasis is significantly influenced by central, humoral, and cognitive inputs that affect energy intake and ultimately balance.

Central Regulation of Energy Homeostasis

Much research has been done to elucidate the component structures and projections that compose the central pacemaker of the human circadian clock. While few direct projections have been established from the SCN to orexin centers, such as the LH, which is important in glucose metabolism (Abrahamson et al, 2001; Yoshida et al, 2006; Sakurai, 2007; Yi et al, 2010), and the VLPO, an important center in regulation of the sleep and wakefulness state, there are many possible indirect projections between these areas (Chou et al, 2002; Yoshida et al, 2006). The SCN modulates food intake by its projections to main brain structures that control food intake including the arcurate nucleus, paraventricular nucleus, lateral and dorsomedial hypothalamic areas (Yi et al, 2006). Knockout of the core clock genes in mice results in metabolic disturbances (e.g. weight gain) though it is unclear whether this is a direct result of clock gene disruption or if it is a downstream effect (Rudic et al, 2004; Turek et al, 2005).

Metabolically relevant peripheral organs such as the liver, pancreas, adrenal cortex and adipose tissue have been shown to receive direct projections from the SCN demonstrating potential avenues for the involvement of the SCN in metabolic function (Buijs et al, 1999; la Fleur et al, 2000; Buijs et al, 2001; Kreier et al, 2006). Furthermore, peripheral organs have cellular circadian clocks influenced by factors other than light exposure (Brown et al, 2002; Schibler et al, 2003; Zvonic et al, 2006; Mohawk et al, 2012). The liver is responsible for

maintaining optimum glucose levels by its role in gluconeogenesis during fasting (Shimazu, 1987; Nonogaki, 2000; Puschel, 2004). Rhythmic fluctuations in plasma glucose (increase in the late afternoon and continued overnight in association with growth hormone) (Van Cauter et al, 1991) are a result of stimulation of liver-dedicated pre-autonomic neurons in the paraventricular nucleus (Kalsbeek et al, 2004; Kalsbeek et al, 2008). Removal of this sympathetic input into the liver eliminated the rhythmic concentrations of glucose in circulation (Cailotto et al, 2005). Rhythmic alterations in glucose concentration could be beneficial allowing for easily accessible energy for muscles during potential times of activity, while maintaining low levels when the probability of activity is low decreases risk of hyperglycemia and associated diseases (Creager et al, 2003).

While light is the strongest zeitgeber for the central clock, it is possible for peripheral organs to integrate their own signaling processes to keep time separate from that of the central pacemaker in the SCN. Food intake is a cue that can impact circadian timing of peripheral organs(Stokkan et al, 2001; Stephan, 2002). Desynchronizing food and light time cues does not change entrainment of SCN electrical activity or clock gene expression (Gooley et al, 2006) suggesting the presence of additional entrainment mechanisms in the periphery. Daily rhythms in liver clock gene expression and plasma glucose concentrations are maintained during fasting (La Fleur et al, 1999; Kita et al, 2002). However, rhythmic gene expression in the liver, kidney and heart are reset by food restriction to the light phase in mice, with the liver entraining to the new time cues faster than the kidney heart or pancreas, leading to internal desynchrony between central and peripheral clocks (Damiola et al, 2000). Interestingly, a high fat diet has been shown to lengthen the intrinsic period length of motor activity, a measure of circadian timing in rodent models. Altering the length of the active period impacted feeding patterns in mice, which was

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shown to shift the phase of metabolic gene expression in peripheral organs including liver and adipose tissue (Kohsaka et al, 2007). This suggests that circadian phase can be influenced by both activity and food intake and can be decoupled from central circadian timing. Whether this desynchrony in turn impacts energy metabolism or subsequent food intake is an important potential connection to weight gain and obesity in circadian and sleep research.

Hormonal Signaling and Energy Metabolism

Hormonal regulation contributes to hunger and food intake. Two major hormones involved in satiety and hunger signaling are leptin and ghrelin. Leptin is released primarily from subcutaneous fat deposits (Ahima et al, 2000) and is a satiety signal. Under constant routine conditions, leptin exhibited a circadian rhythm such that levels increase across the biological day and peak at the end of the biological night (Sinha et al, 1996; Schoeller et al, 1997; Shea et al, 2005). Also leptin levels are increased during daytime sleep compared to daytime wakefulness (Schoeller et al, 1997; Simon et al, 1998) suggesting an impact of sleep on leptin levels. Ghrelin is an appetite stimulating hormone released by stomach cells, which is affected by food intake, exhibiting a preprandial rise and a postprandial fall (Cummings et al, 2001), while leptin is stimulated by food intake (Dallongeville et al, 1998). In fasted individuals and rats, ghrelin continues to rise before habitual meal times and decrease afterward (Natalucci et al, 2005; Drazen et al, 2006). Ghrelin was also found to be higher during the middle of the inactive light phase in nocturnal mice when compared to the middle of the active phase (LeSauter et al, 2009). Timing of leptin and ghrelin sampling with relation to sleep and food intake is an important consideration when interpreting data from these and other studies(Schmid et al, 2008; Omisade et al, 2010; Pejovic et al, 2010; St-Onge et al, 2011). While leptin and ghrelin are not the only

hormonal signals of satiety and hunger in humans and rodents, they are considered primary and provide opportunity for further study in sleep and circadian manipulations.

Another important component of metabolic processes is insulin and glucose function. Reduced insulin sensitivity is a risk factor for developing diabetes and is associated with negative metabolic outcomes (Martin et al, 1992). Insulin is secreted by the pancreas in response to elevated blood glucose levels, which occur after food intake. The primary insulin sensitive tissues are skeletal muscle, adipose and liver as these organs have the highest expression of insulin receptors. Insulin signaling in muscle and adipose tissue causes translocation of the glucose receptor (GLUT4) to the cell membrane resulting in glucose uptake from blood. Blood glucose and insulin levels are positively correlated in normal physiologic function. Glucose and insulin levels vary across 24 hours with glucose concentrations increasing in the late afternoon and remaining high overnight. Insulin secretion is inverse of the cortisol rhythm such that it increases to peak in the middle of the afternoon and reaches a nadir in the second half of the biological night (Boden et al, 1996; Van Cauter et al, 1997b). When the SCN is lesioned in rats this eliminates the 24h rhythmic variation in glucose concentrations (La Fleur et al, 1999). Components of the central circadian clock, CRY1 and CRY2, are rhythmically expressed in the liver which modulates hepatic gluconeogenesis, a method by which glucose can enter the blood stream in times of fasting (Zhang et al, 2010). In addition, melatonin receptors have been discovered on pancreatic beta cells, which are involved in insulin secretion, and melatonin has also been shown to indirectly modulate insulin secretion (Ramracheya et al, 2008; Peschke and Muhlbauer, 2010). Under constant routine conditions, glucose and insulin show circadian rhythm with peak concentrations in the late biological night/early biological morning (Morgan et al, 1998; Shea et al, 2005). Glucose but not insulin demonstrates a circadian rhythm with peak

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concentrations during the biological night under forced desynchrony conditions (Scheer et al, 2009). When constantly infused throughout the day, glucose levels during sleep increase possibly due to decreased brain glucose metabolic requirements associated with sleep (Boyle et al, 1994). Proper response of glucose and insulin to food intake is important to maintaining metabolic homeostasis as dysfunction has been implicated in negative metabolic outcomes. The circadian system and sleep have both been shown to impact normal glucose and insulin function.

Influence of the Circadian System and Sleep on Components of Metabolism

Regulation of metabolic processes including energy expenditure, hormone levels, hunger, and food intake is important for healthy physiological function. Improper function these components can induce positive energy balance, a state which leads to weight gain. Circadian processes and sleep have both been shown to influence many aspects of physiology. Situations of sleep loss or circadian misalignment including shift work and circadian rhythm disorders have demonstrated a connection between the circadian system, sleep, and metabolic health (Depner et al, 2014). The mechanism underlying the influence of the circadian system, sleep and their potential interactions on metabolic dysfunction is currently a topic of great scientific interest.

Circadian Misalignment and Energy Metabolism

Glucose and insulin secretion and signaling have been shown to be influenced by the circadian system. Light exposure at an adverse circadian time, during the normal dark period in rodents, combined with an imbalance in the autonomic inputs to the liver results in a disturbed daily plasma glucose rhythm (Kreier et al, 2003; Cailotto et al, 2005; Cailotto et al, 2008) suggesting circadian disruption can impact glucose concentrations. In addition, *Clock* mutant and

Bmal1 knockout mice, both of which exhibit circadian dysfunction, are hypersensitive to insulin (Rudic et al, 2004) demonstrating involvement of the circadian system in insulin signaling. Under forced desynchrony conditions in humans, Scheer et al (Scheer et al, 2009), glucose was increased by 6% despite a 22% increase in insulin concentration. In this study, subjects were provided four meals per 28-hour day, which were designed to be isocaloric, however, leptin was also found to be decreased and therefore the subjects may not have been in energy balance, which could in turn impact glucose and insulin levels. An additional study, using a forced desynchrony demonstrated an 8% increase in fasting glucose and a 14% increase in postprandial glucose (Buxton et al, 2012). However, plasma insulin during fasting was decreased by 12% as well as 24% after meals. This contradicts previous findings under the same circadian research protocol. In a sleep restriction protocol, insulin sensitivity was decreased by ~20% despite an increase in insulin secretion when tested in the early morning during the biological night after short sleep (Eckel et al, 2015). Night work and circadian misalignment has been shown to decrease leptin levels and impair glucose tolerance and insulin sensitivity (Akerstedt, 1998; Spiegel et al, 1999; Ohayon et al, 2002; Spiegel et al, 2004b; Buxton et al, 2010). In night shift workers tested on shift, postprandial glucose and insulin were both higher when compared to day workers (Lund et al, 2001). Experimentally controlled and observational studies have described altered glucose and insulin levels in cases of circadian misalignment and shift work though there are contradictory findings of insulin response under these conditions.

Signaling of insulin, glucose and other important hormones is only one of the facets influencing energy metabolism. There is a circadian variation in hunger with the peak occurring in the early evening and the trough occurring approximately 12 hours later, in the biological morning (Scheer et al, 2013). Similar circadian rhythms were also shown for foods including

sweet, salty and starchy foods, fruits, meats/poultry, food overall and estimates of how much participants could eat. While there is a decreased circadian drive for hunger, shift workers shift the timing of their meals to occur while they are awake overnight (Reeves SL, 2004; Lowden et al, 2010).The circadian system has also been shown to impact food intake, and the amount and timing of food intake can impact energy balance (Hatori et al, 2012). Exposing mice to 150 lux (normal room lighting) of continuous light during the habitual dark phase significantly increased body and fat mass, and impaired glucose tolerance (Fonken et al, 2010). A greater percentage of food intake also occurred during the light phase. A high-fat diet consumed only during the light phase caused weight gain compared to mice only fed during the dark phase (Arble et al, 2009). When a standard diet is restricted to the light phase, mice increased their initial quantity of food intake after restriction as well as overall calories per day, and exhibited weight gain and diminished amplitude of circadian and metabolic genes in metabolically active tissues including the liver (Bray et al, 2013). However, when mice were restricted to be fed only during the dark phase, their normal active period, they were largely protected against obesity and hyperinsulinemia even when fed a high fat diet (Hatori et al, 2012). Comparison of light-fed, dark-fed, and shift work model with activity during the light phase, both light-fed and shift work model rats demonstrated decreased glucose and locomotor activity rhythms, increased food intake during resting phase and showed subsequent weight gain despite similar caloric intake to dark-fed control rats (Salgado-Delgado et al, 2010). By prohibiting feeding during this time in the dark-fed group, metabolic rhythms were maintained and the rats were protected from weight gain. Circadian timing of food intake has been demonstrated by animal models to impact weight gain even with similar caloric content. Restricting food intake to 9-12 hours of the active phase in humans has been shown to protect against weight gain even on high-fat, high-fructose, and

high-sucrose diets (Chaix et al, 2014). Therefore, education on proper timing of food intake, despite hunger and hormonal signaling for hunger and satiety may represent a potential treatment approach to prevent weight gain and obesity in shift work populations.

Insufficient Sleep and Energy Metabolism

One of the hypothesized functions of sleep is energy conservation. Brain glucose utilization accounts for approximately 50% of daily, whole-body energy use and it is decreased after 24-hours of total sleep deprivation (Thomas et al, 2000). In total sleep deprivation, day time energy expenditure was similar to a day with normal night time sleep but overnight energy expenditure was 32% higher when compared to a night of normal sleep. Quantified, increased metabolic cost of extended wakefulness was 135 kcal on average. Per hour, energy expenditure increased by 17 kcal (Jung et al, 2011). However, when an 8 hour sleep opportunity was timed during the day, energy expenditure decreased by 12-16% (McHill et al, 2014). In another study of partial sleep restriction, 24-hour energy expenditure was increased by 5%, or 111 kcal on average as a result of the energy cost of additional wakefulness (Markwald et al, 2013). In addition, after a night of sleep deprivation, RMR decreased by 5.2% as measured by metabolic chamber (Benedict et al, 2011) and was also decreased by 8% under forced desynchrony conditions (Buxton et al, 2012). When energy expenditure was measured in freely living subjects in other studies, no change in RMR was found (Bosy-Westphal et al, 2008; Nedeltcheva et al, 2009b; Buxton et al, 2010; St-Onge et al, 2011). Interestingly, sleep restriction was shown to increase respiratory quotient (RQ), indicating a greater proportion of energy is being used from carbohydrates than fats (Bosy-Westphal et al, 2008; Hursel et al, 2011). This could suggest that

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under conditions of insufficient sleep, the body switches to more quickly accessible energy from carbohydrates and stores more energy as fat.

Activity levels are also an important component of daily energy expenditure and resulting metabolic homeostasis. While one study reported that physical activity during one afternoon and evening following a night of four hours time in bed was increased compared to following a night of eight hours time in bed (Brondel et al, 2010), findings from the majority of studies show that activity levels are decreased following sleep restriction (Schmid et al, 2009; Booth et al, 2012; Bromley et al, 2012). Additionally, less time was spent engaged in more energy-intensive or vigorous physical activities (St-Onge et al, 2011; Booth et al, 2012; Bromley et al, 2012). Despite the increased energetic cost of wakefulness under sleep restricted conditions, the demonstrated decrease in activity level and intensity can have a potential impact on the overall amount of energy expended and thus energy balance, increasing the potential for weight gain.

In conjunction with energy expenditure, there are other regulatory controls of energy balance. Insulin and glucose are important in maintaining energy homeostasis as dysregulation in either or both of these systems has been associated with metabolic disease. Sleep restriction has been shown to decrease insulin sensitivity by as much as 18-24%, impair insulin response and decrease glucose tolerance (Spiegel et al, 1999; Spiegel et al, 2004b; Nedeltcheva et al, 2009a; Buxton et al, 2010; Leproult and Van Cauter, 2010; Broussard et al, 2012; Klingenberg et al, 2013). Suppression of slow wave sleep also resulted in a decrease in insulin sensitivity (Bergman, 1989; Stamatakis and Punjabi, 2010) without an adequate compensatory increase in insulin (Tasali et al, 2008). Interestingly, these metabolic consequences of sleep restriction were at least partially reversible with recovery sleep as evidenced by improved glucose tolerance (Spiegel et al, 1999) and a reduction in the insulin-to-glucose ratio (van Leeuwen et al, 2010).

Under conditions of sleep restriction, the body's physiology does not properly respond to regulate glucose and insulin, indicating that food intake and short sleep could predispose the system to metabolic dysregulation.

Hunger and satiety hormones including ghrelin and leptin play an important role in regulating food intake and subsequent energy balance. Ghrelin levels have been shown to increase in the early part of sleep and in sleep deprivation this response is blunted (Dzaja et al, 2004). Total sleep deprivation resulted in increased ghrelin (Benedict et al, 2011) while sleep restriction has been associated with a reduction in total and acylated ghrelin (Dzaja et al, 2004; Buxton et al, 2012) and an increase in total ghrelin levels (Spiegel et al, 2004b; Taheri et al, 2004) when energy balance conditions are not known. Sleep loss can increase ghrelin when subjects are also in negative energy balance (Buxton et al, 2012; Kilkus et al, 2012; Penev, 2012). However, under conditions of positive energy balance, sleep loss shows no change in total ghrelin levels (Bosy-Westphal et al, 2008; Nedeltcheva et al, 2009a; Schmid et al, 2009; Markwald et al, 2013). Similarly, in studies involving ad libitum feeding which often is associated with positive energy balance, no changes in leptin or ghrelin are observed (Bosy-Westphal et al, 2008; Nedeltcheva et al, 2009a; Schmid et al, 2009; Omisade et al, 2010; Pejovic et al, 2010; Simpson et al, 2010; Markwald et al, 2013). Another study found no association between leptin levels and sleep duration, efficiency, or disturbance using actigraphy (Knutson et al, 2011). However, after one night of total sleep deprivation compared to baseline, average leptin was found to be increased (Pejovic et al, 2010). When food intake was controlled, morning but not evening leptin levels were found to be increased after one night of 3 hours time in bed (Omisade et al, 2010). Morning leptin levels were also increased after 5 nights of 4 hours time in bed when compared to 2 nights 10 hours time in bed with self-selected food intake

(Simpson et al, 2010). In studies of sleep restriction, each hour of sleep loss increased leptin levels by 6%, when controlling for age, gender, race, BMI (Hayes et al, 2011). Leptin levels were only found to be decreased after 6 nights of 4 hours time in bed compared to 12 hours time in bed (Spiegel et al, 2004a). Though there is conflicting evidence on how hunger and satiety hormones are responding to sleep loss in the presence of altered feeding patterns, this system exhibits potential sensitivity to changes in sleep duration which can disrupt hormonal signaling.

While energy balance and hormonal signaling are important aspects of metabolic functioning, cognitive function as assessed by hunger ratings plays an important role as well. Total sleep deprivation does not show a difference in hunger ratings compared to a normal night of sleep (Pejovic et al, 2010). Acute sleep restriction (1-4 nights of >4-hours time in bed) did not change hunger ratings compared to baseline sleep in both men and women (Bosy-Westphal et al, 2008; Schmid et al, 2009; Omisade et al, 2010; St-Onge et al, 2011) (men only). Men reported higher hunger ratings after sleep deprivation (Schmid et al, 2008) and sleep restriction (Spiegel et al, 2004b), with the most marked increase in appetite being associated with calorie-dense, high carbohydrate foods (Spiegel et al, 2004b). These two studies that reported a difference in hunger ratings included only young, male participants. In another study of sleep restriction, men had lower subjective ratings of fullness compared to normal sleep and there was a sex by sleep interaction as these effects were not seen in women (St-Onge et al, 2011). In addition, poor sleep quality but not sleep duration was associated with increased hunger (Kilkus et al, 2012) especially after dinner (Gonnissen et al, 2013). It has also been demonstrated in insufficient sleep that increases in ghrelin and decreases in leptin consistent with the hormonal signal for food intake presents concurrent with increased hunger ratings (Taheri et al, 2004; Nedeltcheva et al, 2009a), especially for calorie-rich, high carbohydrate foods (Spiegel et al, 2004a; Spiegel et al,

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2004b; Benedict et al, 2011).This demonstrates that in certain populations under conditions of insufficient sleep, cognitive function as assessed by subjective hunger ratings can be altered.

Cortical activation in response to food stimuli has also been studied as a method for understanding physiological responses involved in energy homeostasis. Sleep deprived subjects showed decreased activity in the frontal and insular cortices, regions that are sensitive to appetite, and increased amygdala activity when asked to rate food desirability (Greer et al, 2013). However, reward and food-sensitive regions showed increased responsivity when presented with unhealthy food stimuli during sleep deprivation (St-Onge et al, 2012b). In sleep restriction, food-sensitive and reward areas showed increased activation in response to presentation of food stimuli(Benedict et al, 2012; St-Onge et al, 2012a; St-Onge et al, 2014), with the insular cortex, orbitofrontal cortex and dorsolateral prefrontal cortex displaying strongest activation in response to unhealthy food compared to healthy food stimuli (St-Onge et al, 2014). Taken together, the implications of both the hunger/appetite and neuronal activation cognitive data suggest that there are effects of insufficient sleep on cognitive functioning. It is possible that the hedonic factors associated with food intake, such as increased subjective reward or pleasure derived from the food choice, could be preferentially in decision making processes used when sleep is not sufficient.

Cognitive function, energy balance and hormonal signaling have been demonstrated to be altered under conditions of insufficient sleep. These findings have implications for how and when food is chosen. However, they are not sufficient to understand the metabolic consequences of sleep loss. The action of food intake is a critical portion of energy balance equation that determines metabolic outcomes. Insufficient sleep has been associated with increase caloric intake up to ~559 kcal when food is presented ad libitum (Bosy-Westphal et al, 2008;

Nedeltcheva et al, 2009a; Brondel et al, 2010; St-Onge et al, 2011; Chapman et al, 2012;

Beebe et al, 2013; Calvin et al, 2013; Markwald et al, 2013; Spaeth et al, 2013; Chaput, 2014).

Timing of food intake is a critical component of overall food intake, as total sleep deprivation

did not increase buffet food intake in the late afternoon (Benedict et al, 2011). Short sleep has

also been associated with poor diet quality, including low fruit and vegetable consumption

(Stamatakis and Brownson, 2008), increased fat intake (Brondel et al, 2010; Nishiura et al,

2010; St-Onge et al, 2011), greater frequency of restaurant or fast food consumption (Weiss et

al, 2010), increased intake of calories from snacks (Kim et al, 2011) especially carbohydrates

(Nedeltcheva et al, 2009a). In sleep restriction, food intake is also timed later with increased

calorie intake during the overnight hours (Spaeth et al, 2013). Decreased percent of calories at

breakfast and increased calories consumed after dinner as snacks containing fats and

carbohydrates has also been observed under sleep restricted conditions (Markwald et al, 2013).

Interestingly, these changes in food intake occur with appropriate responses of hunger hormones

to excess food intake (Nedeltcheva et al, 2009a; Markwald et al, 2013), suggesting that the

hedonic valuation of food during sleep deprivation can overcome homeostatic and hormonal

signals for food intake.

Growth Hormone

Growth hormone (GH) is an endocrine hormone which has a strong sleep-related

secretory pattern. It is a component of the somatotropic axis and is involved in growth and

development in early years but is released throughout life and is involved in anabolic protein

metabolism as well as energy metabolism.

Endocrine Control

GH is a peptide hormone secreted by cells in the anterior pituitary. Synthesis and

secretion of GH is stimulated by growth-hormone releasing hormone (GHRH) and inhibited by

somatostatin. Both are hypothalamic neurohormones, secreted into the hypothalamic-

hypophyseal system and transported to their receptor sites in the anterior pituitary. Interestingly,

ghrelin, a hormone previously mentioned, can also stimulate the secretion of GH. Though it is

primarily recognized for its anabolic functions as a trophic hormone to stimulate the secretion of

insulin-like growth factors (IGF), GH activity also significantly impacts energy metabolism.

Normal metabolic function prioritizes glucose and protein catabolism to obtain the energy

necessary to carry out necessary functions. In the presence of GH, metabolism shifts to favor

lipolysis to spare glucose and protein stores, decreasing hepatic glucose output and increasing

free fatty acids (FFAs) (Rabinowitz and Zierler, 1963; Moller et al, 1990). GH and IGF are

symbiotic and work to regulate energy availability by stimulating the appropriate catabolic

processes. IGF is produced in the liver and in the presence of sufficient nutritional intake and

elevated insulin, its release is stimulated by GH (Wurzburger et al, 1993) and promotes protein

anabolic processes regulated by GH (Clemmons and Underwood, 1991). In the occurrence of

extending fasting, GH concentrations increase and IGF concentrations decrease, favoring

lipolysis (Moller et al, 1990). The presence of GH has also been linked to decreased glucose

uptake by muscle tissue (Zierler and Rabinowitz, 1963) and decreased insulin sensitivity

(Djurhuus et al, 2004). Interestingly, GH levels are also seen to be increased in Type 1 Diabetes

patients (de Sa et al, 2010). These functional aspects of GH demonstrate a physiologically

adaptive mechanism for increased GH secretion during the night to decrease glucose uptake

during overnight fasting as well as potential promotion of anabolic processes at a time when

other energy demands are not as high and that dysregulation can negatively impact energy metabolism.

Sleep and Circadian Regulation of GH

Growth hormone is secreted in a pulsatile manner with a large pulse occurring in the early night (Takahashi et al, 1968; Honda et al, 1969). Other pulses have been observed ~4h after meal consumption (Ho et al, 1988; Hartman et al, 1991), consistent with previously mentioned connections to energy metabolism and fasting metabolic regulation. The nighttime GH pulse can account for over 50% of the total GH secretion over 24h (Van Cauter et al, 1998), indicating that the sleep-GH relationship is physiologically significant and merits investigation. This large pulse of GH secretion has been associated with the first entry into NREM Stage 3/4 (SWS), which occurs within the first REM cycle (Honda et al, 1969; Sassin et al, 1969b). The amount of GH secreted in this pulse has been correlated with the duration (Holl et al, 1991; Van Cauter et al, 1992) and intensity (Gronfier et al, 1996) of SWS. Sampling of plasma for GH every 30s has been able to show with great temporal precision that maximal levels of GH secretion are achieved within minutes of SWS onset (Holl et al, 1991). An additional study demonstrated that ~70% of GH pulses during sleep occurred in NREM SWS (Van Cauter et al, 1992). Together, these findings demonstrate strong evidence for the temporal correlation of the secretion of the majority of daily GH and the occurrence of NREM SWS, though GH secretion does occur at other times of sleep and wakefulness.

Pharmacological enhancement of SWS has been shown to increase GH secretion in a dose-dependent manner (Gronfier et al, 1996; Van Cauter et al, 1997a), while pharmacological SWS inhibition was associated with decreased GH (Seifritz et al, 1995). Behavioral deprivation

or disturbance of SWS also decreases GH release (Sassin et al, 1969a; Karacan et al, 1971; Beck, 1981). Additionally, afternoon naps show increased GH secretion and SWS compared to naps earlier in the day, which contain more REM sleep due to circadian influence (Karacan et al, 1974; Othmer et al, 1974) providing further evidence in support of the correlation between SWS and GH secretion. There is, however, mixed evidence of the effects of GH infusion on sleep architecture (Mendelson et al, 1980; Kern et al, 1993), demonstrating that GH itself may not be impacting SWS but that it may be SWS permitting the secretion of GH. Timing of administration may also play a role in the GH/SWS response, as identical doses of growth-hormone releasing hormone (GHRH), which simulates GH secretion, administered at 3h intervals across the 24h day showed increased GH release triggered in the early evening (Jaffe et al, 1995). Additionally, when GHRH bolus is administered at the start of SWS the resulting GH secretion is increased in amount and duration compared to a GHRH pulse given during the waking day (Van Cauter et al, 1992). Together this evidence provides strong support for the mechanistic and temporal link between GH and related hormones and SWS.

Impact of Sleep and Circadian Research Protocols on GH

As GH is seen to be closely related to sleep architecture, sleep and circadian research techniques have been employed to investigate the effect of changes on the timing and amount of GH secretion. Following >24h of total sleep deprivation (TSD), studies have shown that GH secretion is significantly reduced during evening wakefulness and that a normal large GH secretion pulse returns during the subsequent recovery sleep (Davidson et al, 1991; Van Cauter et al, 1991). GH secretion during sleep has also been examined using a variety of sleep restriction manipulations. Largely, sleep restriction has shown that the GH secretory pattern is

conserved, or the largest GH pulse occurred during sleep in cases of a 3h (Honda et al, 1969), 5h (Van Cauter et al, 1992) and 8h (Weibel et al, 1997) delay. However, it is important to note that evidence from sleep restriction studies demonstrates that not all GH secretion co-occurs with SWS. Specifically, GH shows a minor increase prior to sleep onset in the late evening of sleep restriction (Mendlewicz et al, 1985; Steiger et al, 1987; Jarrett et al, 1990; Mullington et al, 1996). This conservation of some GH increase in the early night suggests that an interaction may exist between the circadian and sleep systems to allow for maximal GH secretion to occur in the early part of the sleep episode. The impact of the circadian system via circadian misalignment on GH has also been tested. One study employing 3h sleep periods rotated across the circadian cycle has demonstrated that GH levels following sleep onset during sleep periods occurring in the late evening (i.e. near habitual bed time/beginning of the biological night) had significantly increased GH secretion after sleep onset than episodes occurring at other times of the day and night (Weitzman et al, 1974; Aschoff, 1978). Additionally, in subjects who were permitted to self-select sleep and wakefulness timing in the absence of environmental time cues, who became desynchronized from the 24h day and moved to a free-running circadian timing pattern, peak GH secretion was decreased during the free-running period compared to baseline but average secretion remained unchanged. These alterations were found concurrent with changes in SWS (Weitzman et al, 1981; Moline et al, 1986). It is evident from these findings that the circadian system does play a role in GH secretion.

Summary

This review has discussed the physiology and function of circadian and sleep systems, as well as metabolic and endocrine processes. Furthermore, this information highlighted the

integration of these processes and implications for circadian misalignment and healthy physiological function. Circadian biology and sleep physiology function together to regulate many important process for maintenance of healthy function. As altered sleep and wakefulness schedules have increased in modern society, investigation of the contributions of these processes to metabolic health and pathogenesis, in the form of cardiovascular disease, diabetes, weight gain and obesity, is essential. Further investigation of the physiological and behavioral impact of circadian misalignment as it plays into these conditions is important for the development of strategies and countermeasures to address negative health outcomes.
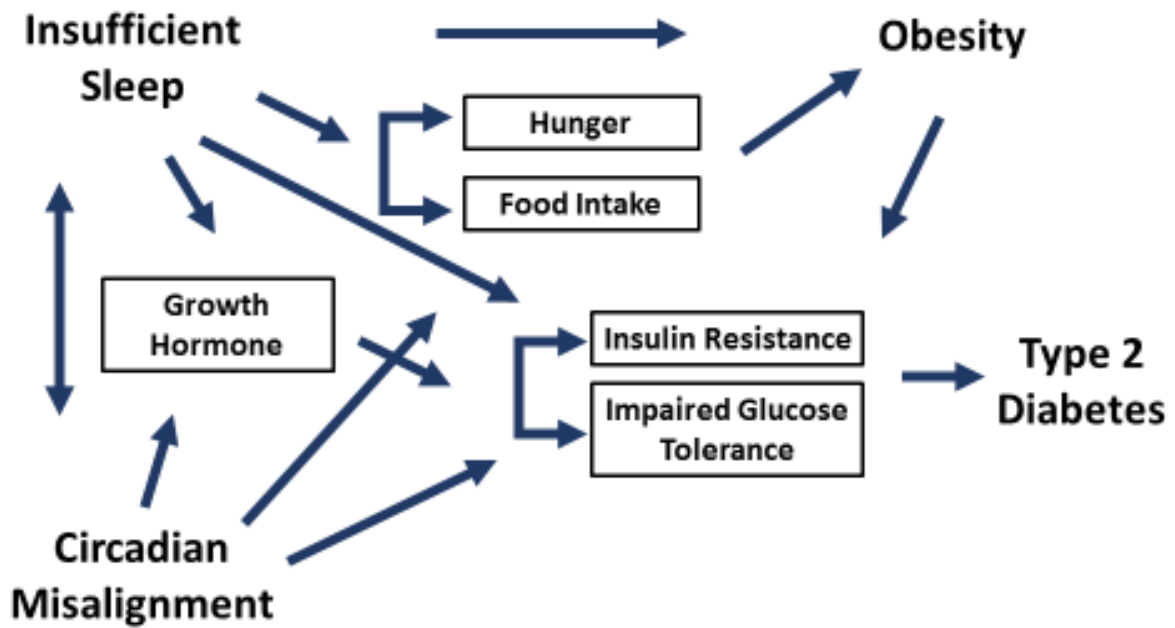
Figure 1. Proposed Model of the Impact of Circadian Misalignment and Insufficient Sleep on Altered Metabolism Leading to Weight Gain and Obesity

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CHAPTER 2

EARLY MORNING FOOD INTAKE AS A RISK FACTOR FOR

METABOLIC DYSREGULATION

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ABSTRACT

OBJECTIVE: Increased risk of obesity and metabolic diseases in shift workers may be related to food intake at adverse circadian times. Early morning shiftwork represents the largest proportion of shift workers in the USA, yet little is known about the impact of food intake in the early morning on metabolism.

METHODS: Twenty-two subjects (12 female) aged 23.0 ± 3.5y ($\pm$SD) BMI 23.5 ± 2.0 completed a 16-day counterbalanced protocol. Subjects maintained habitual sleep one-week prior to each of two study visits: 8h sleep opportunity at habitual time; simulated early morning shiftwork with 6.5h sleep opportunity starting ~1h earlier than habitual. After waking, blood was sampled for fasting glucose, insulin, and melatonin. An identical breakfast was then served in each condition and blood samples were collected every ~40min for 2h.

RESULTS: Simulated early morning shiftwork decreased total sleep time and induced morning circadian misalignment. Glucose levels were ~5% higher following breakfast during circadian misalignment versus habitual sleep ($p<0.05$). Resting energy expenditure was initially lower ($p<0.01$) during simulated early morning shiftwork, whereas the thermic effect of food was similar between conditions ($p=0.21$).

CONCLUSIONS: Circadian misalignment and food intake during early morning shiftwork may contribute to metabolic dysregulation.

INTRODUCTION

Metabolic diseases, including obesity and diabetes, continue to increase in prevalence (1). An estimated 65% of working age adults in the USA are affected by overweight and obesity and over 9.3% by diabetes (2). Such diseases are associated with an increased risk of hypertension (3), cardiovascular diseases (4), and hyperlipidemia (5). Insufficient sleep is a novel risk factors that contributes to weight gain and type 2 diabetes (6). This may in part be due to the consumption of food during the biological night (7), a time when physiological processes are not prepared for food intake. Findings from studies of nocturnal animals have shown that food intake during the typical inactive period and leads to impaired glucose tolerance and weight gain (8, 9, 10). However, restricting food intake to the habitual active phase has been shown to maintain normal glucose tolerance and protect animals from excessive weight gain seen with a high fat diet (9).

In humans, shift workers are chronically exposed to altered behavioral schedules of sleep and wakefulness that result in insufficient sleep, circadian misalignment and food intake during the internal biological night. Circadian misalignment during simulated nightshift and work has been shown to impair glucose tolerance acutely and immediately upon return to simulated day work (11, 12). Most research on the metabolic effects of shiftwork has focused on the overnight hours, however, individuals who begin work in the early morning [between 04:00 and 07:00, (13)] make up the largest population of shift workers in the United States (14). Early morning shiftwork requires awakening earlier than on days off, which would be expected to induce wakefulness during the biological night when endogenous melatonin levels are high–morning circadian misalignment. Additionally, sleep prior to early morning shiftwork is often shortened (15), as it requires an early morning awakening and since it can be difficult to go to bed earlier

prior to an early shift during the circadian wake-maintenance zone in the evening hours (16). Early morning shiftwork has been associated with higher levels of fasting insulin resistance (HOMA-IR) (17). Based on these findings, it is possible that food intake during early morning shiftwork may contribute to negative metabolic outcomes, which was the focus of the current study.

METHODS

Subjects

Twenty-two subjects (12 female) aged 23.0 ± 3.5y ($\pm$SD) BMI 23.5 ± 2.0 participated in the 16-day protocol. Data from one female subject is not included as blood samples were unable to be obtained on one visit and three (2 female) subjects completed only one visit. Thus, 18 subjects contributed to the final analysis. Study procedures were approved by the University of Colorado Boulder Institutional Review Board. Subjects gave written informed consent at the Sleep and Chronobiology Laboratory and were compensated for their participation. Subjects reported being free from any current medical or psychiatric diagnosis, medications, drugs, and were non-smokers. Subjects were healthy as assessed by physical, psychological and sleep disorders screenings. To determine health status, physical exam, blood chemistries, 12-lead clinical electrocardiogram, and urine toxicology were performed at the Clinical and Translational Research Center at the University of Colorado Boulder. Prior to study, subjects did not participate in shiftwork for six months or travel more than one time zone for three weeks.

Protocol

Each subject completed two counterbalanced study conditions (Figure 1), a habitual sleep condition and a simulated early morning shiftwork condition in a crossover design, with half of the subjects starting in the early morning shiftwork condition first; equal for men and women. For one week prior to each visit, subjects maintained consistent, habitual, self-selected 8h sleep schedules. Adherence was verified via sleep-wake logs, call-ins to a time-stamped voice-recorder, and wrist actigraphy. Caffeine and alcohol use was also proscribed during this time. Urine toxicology and breath alcohol testing were performed upon admission to the laboratory to verify that subjects were free of these substances. Females also completed a urine pregnancy test at the medical screening and upon admission. For three days prior to the laboratory protocol, subjects consumed an energy-balanced diet, determined by resting metabolic rate (RMR x and activity factor 1.5; Parvomedics) assessment and refrained from physical activity during this time. Timing of sleep and study procedures, including food intake, was scheduled relative to each subject's habitual sleep timing to maintain relative consistency with individual circadian timing.
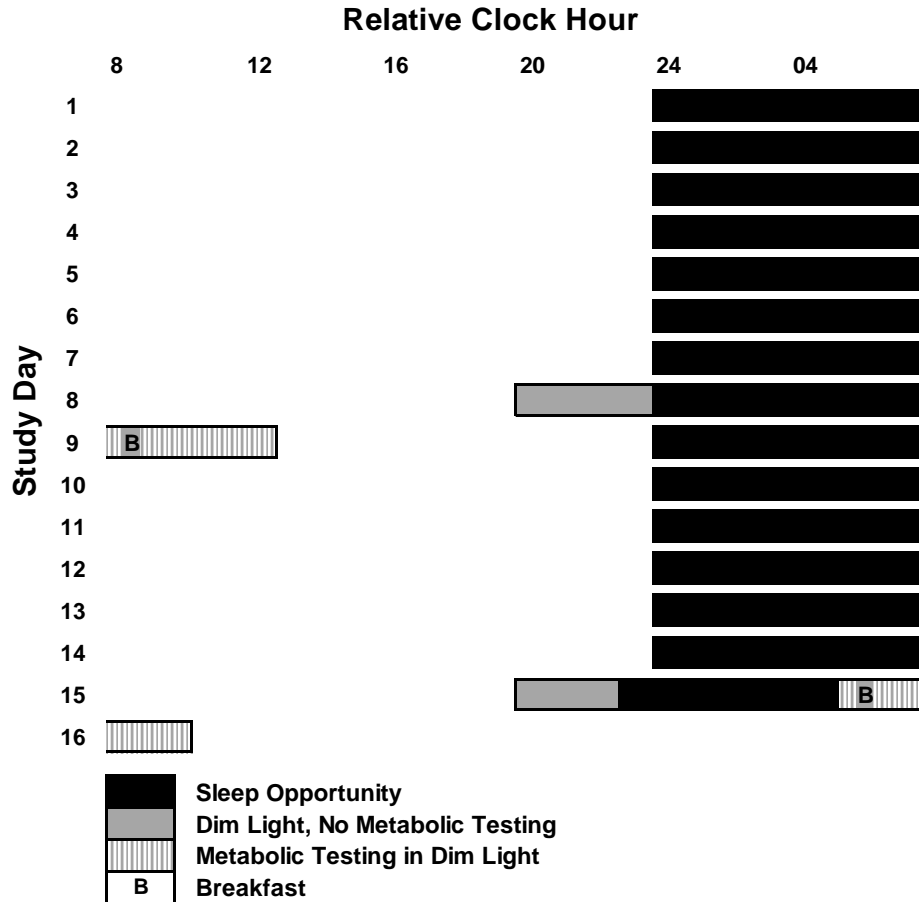
Figure 1. Example Study Protocol. 16-day study protocol for a subject with a habitual sleep schedule of 00:00-08:00. 8h self-selected sleep schedules maintained at home are represented by black bars on days 1-7 and 9-15. Subjects arrived to the laboratory on the evening of the 8th and 15th day of the study, were acquainted with study procedures and instrumented (represented by the gray bars). Subjects then were scheduled to sleep for either 8h at their habitual time as a control condition or for 6.5h that began 1h prior to habitual bed time and ended 2.5h prior to habitual wake as a simulated early morning shiftwork condition. For each visit, subjects completed initial metabolic testing and blood samples were taken for metabolic and circadian markers (represented by the lined bars) after waking. Subjects were then served an identical breakfast at the same time after waking in each condition (represented by "B") and post meal testing continued for ~3h (represented by the lined bars).

Subjects arrived at the laboratory ~4h prior to their scheduled bedtime to be acquainted with laboratory procedures and prepared for polysomnography sleep recordings. In the habitual sleep condition, subjects were given an 8h sleep opportunity at habitual time. The simulated early morning shiftwork condition was designed based on unpublished data from early morning shift workers collected by our laboratory. In this condition, subjects were given a 6.5h sleep opportunity scheduled from ~1h prior to habitual bedtime until ~2.5h prior to habitual waketime. In both conditions after waking, subjects remained in dim light, seated in bed, semi-reclined, with the head of the bed raised to ~35°. Blood was collected for fasted glucose, insulin, and melatonin levels and baseline RMR testing was completed to assess fasted resting energy expenditure (REE). Subjects were then served breakfast scheduled at 45min after awakening in each condition which was identical within subjects and consisted of 25% of individual daily caloric needs: 30% fat, 55% carbohydrate, 15% protein. Following the meal, blood was then sampled every ~40 min for the next 2h and RMR was completed every ~45min for ~3h to assess diet-induced thermogenesis (DIT; the amount of energy needed to process food, absorb, and store nutrients following food intake).

Biological Specimen Analyses

Melatonin, insulin, and glucose levels were assessed in blood plasma drawn from an indwelling catheter placed upon scheduled wake time. Blood samples were processed immediately, centrifuged and frozen at -70ºC until assayed. Melatonin was measured by the Sleep and Chronobiology Laboratory using radioimmunoassay (RIA; Rocky Mountain Diagnostics, Colorado Springs, CO). Insulin and glucose were assayed by the Colorado Clinical and Translational Sciences Institute (CCTSI) Core Laboratory. Insulin was measured by RIA

(Millipore) and glucose was assayed using hexokinase, UV (Beckman Coulter). Assay sensitivity, specificity and coefficients of variation can be found in Supporting Information.

Sleep Analyses

Sleep and wakefulness recordings, including sleep disorders screen, were obtained using Siesta digital recorders (Compumedics). Sleep criteria were defined according to standard guidelines (18).

Data and Statistical Analyses

Mixed model ANOVA (STATISTICA, StatSoft) was used to examine changes in melatonin, insulin, glucose, and RMR outcomes with condition and sample time as fixed factors and subject as a random factor. Sex and order were initially included in models to test impact on variables of interest and none were significant, thus were removed for final analyses. Planned dependent t-tests for individual time points were used to examine differences between conditions. DIT was calculated as deviation from fasted REE measured upon waking prior to meal (19).

RESULTS

Circadian Melatonin Rhythm and Sleep Staging

Melatonin levels were elevated in the simulated early morning shiftwork compared to the habitual sleep condition for the two hours measured after awakening (Figure 2; condition x time interaction, $p<0.0001$). In addition, subjects slept an average of 350.7 ± 4.1 min in the simulated early morning shiftwork compared to 435.8 ± 5.8 min in the habitual sleep condition ($p<0.0001$;

Table S1). This reduction in total sleep time (TST) is a result of decreased time spent in Non-Rapid Eye Movement (NREM) Stage 2 and Rapid Eye Movement (REM) sleep (both $p<0.0001$; Table S1). No differences were found in time in NREM Stages 1 or 3/4 (both $p>0.11$). Additionally, there was no difference in sleep efficiency (SE), sleep onset latencies (SOL), wakefulness after sleep onset (WASO), or the number and duration of awakenings between conditions (all $p>0.14$; Table S1).

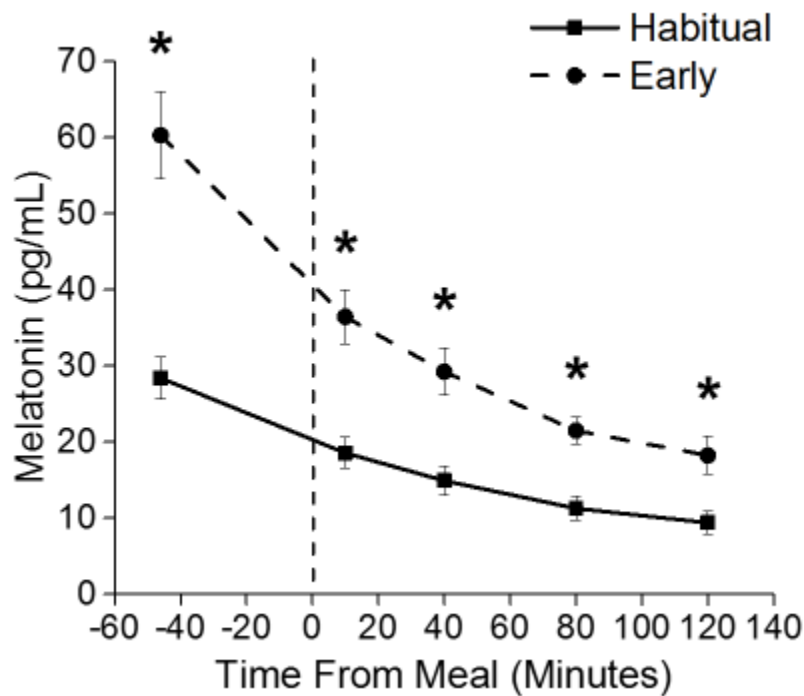**Figure 2. Melatonin Levels.** Melatonin levels from both the habitual sleep (squares) and early morning shiftwork (circles) conditions. The dashed line represents scheduled meal time. * denotes $p<0.05$ between habitual sleep and early morning shiftwork conditions. Average melatonin levels are significantly higher at each time point after waking in the early morning shiftwork condition compared to the habitual sleep condition

Glucose and Insulin Levels

  Glucose levels were elevated by ~5% in the simulated early morning shiftwork compared to the habitual sleep condition after awakening (main effect of condition, $p < 0.05$; Figure 3), whereas insulin levels were similar between conditions ($p > 0.30$).
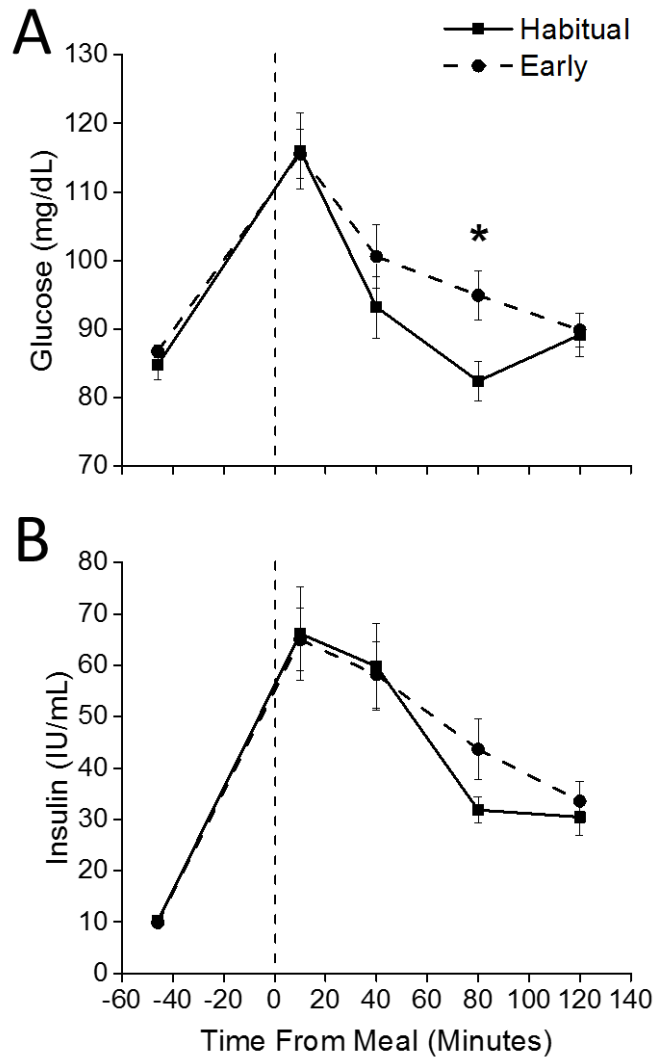

Figure 3. Insulin and Glucose. Plasma glucose (A) and insulin (B) levels fasted and in response to an identical breakfast meal within subjects in both habitual sleep (squares) and simulated early morning shift work (circles) conditions.

Energy Expenditure and Thermic Effect of Food

Energy expenditure was initially lower during the simulated early morning shiftwork condition (condition x time interaction, p<0.01); however, none of the planned comparisons between conditions at individual time points were significant (all p>0.13; Figure S1). Additionally, DIT was not different in response to the meal eaten at an earlier circadian time in the simulated early morning shiftwork condition compared to the habitual sleep condition (6.3%±1.0% habitual sleep and 8.1%±1.1% for simulated early morning shiftwork; p=0.21; Table S1).

DISCUSSION

Obesity and diabetes are associated with negative long-term health outcomes and are highly prevalent among the general population. Shift workers have an increased risk for developing these diseases but the mechanisms underlying this increased risk are unclear. One potential risk factor is food intake at an adverse circadian time, such as during the biological night. Our findings show that food intake after waking in the simulated early morning shiftwork condition elevated plasma glucose without a compensatory insulin response when compared to the same food intake after habitual sleep. Additionally, energy expenditure was initially lower upon awakening in the simulated early morning shiftwork condition but EE and DIT were both similar following food intake.

The current study was designed based on preliminary observational data from early morning shift workers and aimed to simulate concurrent sleep restriction and circadian misalignment that occurs in this work schedule and examine the metabolic consequences. In the simulated early morning shiftwork condition, subjects slept ~85 min less and had higher average

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melatonin levels for the ~2h after waking compared to the habitual sleep condition. This is consistent with other studies showing decreased sleep duration in morning shiftwork (15). The findings of decreased sleep and increased melatonin levels after waking in the early morning shiftwork condition demonstrate that our protocol successfully induced sleep restriction as well as morning circadian misalignment in the simulated early morning shiftwork condition.

Acute exposure of healthy young adults to one night of simulated early morning shiftwork increased glucose levels by ~5% which is similar to that found in other circadian misalignment protocols using simulated night shiftwork models (11, 20). The postprandial glucose levels observed did not meet clinical criteria for impaired glucose tolerance but does potentially indicate decreased insulin sensitivity. Additional research is needed to assess metabolic outcomes following chronic early morning shiftwork, using habitual early morning shift workers, as well as including the use of more sensitive tests, such as oral (OGTT) or intravenous (IVGTT) glucose tolerance tests and hyperinsulinemic/euglycemic clamps.

Energy expenditure is an important component in weight maintenance, including 24h energy expenditure, resting energy expenditure (REE) and diet-induced thermogenesis (DIT). Energy expenditure was initially lower after waking early in the simulated early morning shiftwork condition but increased after food intake to become similar between conditions. Additionally, DIT was not different after the same meal was consumed in simulated early morning shiftwork compared to habitual sleep. The thermic effect of food has been shown to be decreased in response to a meal in the night (8PM – 1AM) compared to the morning (8AM – 9AM) (21, 22). These findings could be due to the influence of circadian phase, though we did not find an impact of circadian phase on DIT in our study. This could be due to a smaller difference in circadian timing between conditions in our study compared to previous studies.

This does not indicate that decreased energy expenditure in response is likely a mechanism for weight gain seen in shiftwork.

Our findings have potential implications for the metabolic health of early morning shift workers and indicate the need to test whether delaying breakfast intake after early morning awakening until habitual breakfast time on days off improves the metabolic health.

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CHAPTER 2

SUPPLEMENTARY MATERIALS

EARLY MORNING FOOD INTAKE AS A RISK FACTOR FOR

METABOLIC DYSREGULATION

Supplemental Table S1

Supplemental Figure S1

SUPPLEMENTAL METHODS

Biological Specimen Analyses

Plasma melatonin radioimmunoassay (RIA): sensitivity 2.3 pg/mL; intra- and interassay coefficients of variation 11.0% and and 10.7%, respectively (Rocky Mountain Diagnostics, Colorado Springs, CO). Insulin RIA: sensitivity 3 uU/mL; intra- and inter-assay coefficients of variation 5.2% and 9.8%, respectively (Millipore). Glucose hexokinase, UV: sensitivity 10 mg/dL; intra- and interassay coefficients of variation 0.67% and 1.44%, respectively (Beckman Coulter).

Sleep Analyses

Sleep onset latency, was scored as time from lights out to the onset of three continuous epochs (SOL 1.5min) or twenty continuous epochs (SOL 10min) of PSG-defined sleep; Wakefulness after sleep onset (WASO), minutes of wakefulness after SOL 1.5min, respectively. Latency to rapid-eye movement (REM) and slow-wave (SWS) sleep from SOL 1.5min was also scored.

SUPPLEMENTAL TABLE/FIGURE LEGENDS

Table S1

Parameter	Habitual Sleep	Simulated Early Morning Shiftwork	P Value
Percent of Recording Time			
Stage 1	3.4 ± 0.3	3.8 ± 0.4	0.39
Stage 2	49.1 ± 1.1	46.1 ± 2.2	0.18
Stage 3/4 (SWS)	16.0 ± 1.4	21.5 ± 2.0	0.0003*
REM	22.4 ± 1.0	17.3 ± 0.9	0.00002*
Minutes of Recording Time			
Stage 1	16.3 ± 1.7	15.2 ± 1.6	0.63
Stage 2	235.4 ± 5.0	182.3 ± 8.9	0.000006*
Stage 3/4 (SWS)	76.9 ± 6.9	84.8 ± 7.9	0.11
REM	107.3 ± 4.6	68.4 ± 3.6	0.000001*
Total Sleep Time (TST)	435.8 ± 5.8	350.7 ± 6.0	0.000001*
Sleep Efficiency (SE)	90.8 ± 1.2	88.7 ± 1.0	0.14
SOL 1.5 min	16.3 ± 3.3	16.4 ± 2.6	0.97
SOL 10 min	17.9 ± 3.3	19.2 ± 2.8	0.77
WASO from SOL 1.5min	27.9 ± 4.1	28.3 ± 4.3	0.94
REML from SOL 1.5min	109.7 ± 9.6	95.5 ± 9.0	0.33
SWSL from SOL 1.5min	15.2 ± 1.5	14.0 ± 1.7	0.39
No. of Awakenings	21.1 ± 2.1	18.5 ± 2.0	0.19
Avg Duration of Awakenings	1.3 ± 0.2	1.5 ± 0.2	0.60

Supplemental Table S1. Sleep Architecture. Findings for changes in sleep quality between simulated early morning shift work and habitual sleep timing are described in main text. Data are presented ± SEM. Abbreviations are designated as follows: Slow wave sleep (SWS); Rapid eye movement (REM); Sleep onset latency (SOL); Wakefulness after sleep onset (WASO); Latency to REM Sleep (REML); Latency to SWS (SWSL).
*Denotes $p < 0.05$ between sleep in habitual condition and sleep in simulated night shiftwork condition.

Figure S1
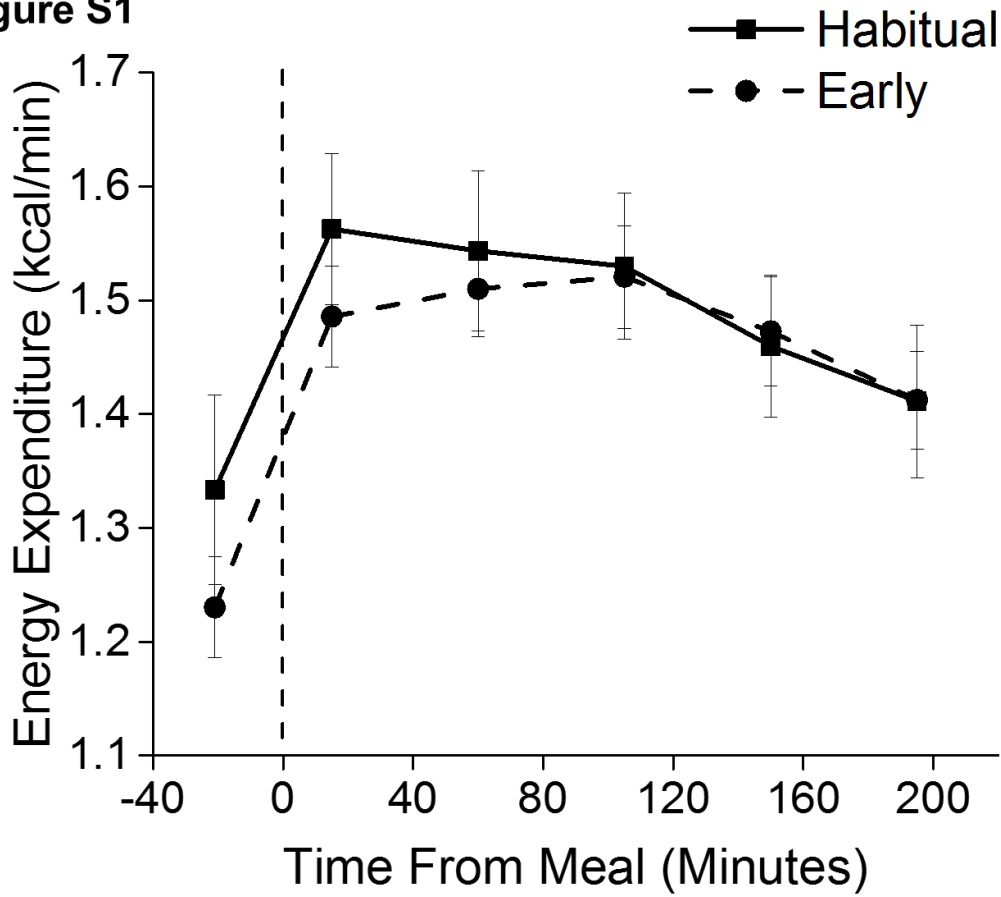
Supplemental Figure S1. Energy expenditure. Energy expenditure was assessed upon waking (fasted) and for 3.5h after identical test breakfast in both habitual sleep (squares) and simulated early morning shiftwork (circles) conditions. The dashed line represents scheduled meal time. Findings are described in main text.

CHAPTER 3

HUNGER AND APPETITE RATINGS DURING

CIRCADIAN MISALIGNMENT AND SLEEP DEPRIVATION

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ABSTRACT

Metabolic diseases such as weight gain and obesity are increasing in modern society. Shift workers are especially effected, potentially due to altered sleep and wakefulness schedules impacting the timing of food intake. However, the influence of the circadian system on appetite with and without food intake at night is largely unknown. The influence of feeding versus fasting at night on appetite for a variety of foods and overall hunger ratings were examined in two protocols: Protocol one simulated night shiftwork with sleep during the day and wakefulness, including meals at night. Protocol two was total sleep deprivation with no food consumed at night. A total of twenty-one healthy adults participated. Fourteen adults (n=6 males) aged (26.4 ±1.2 y), BMI (22.7 ±0.5 kg/m^2) participated in the simulated shiftwork protocol (McHill et al, 2014) and seven adults (n=5 males) aged (22 ±5 y), BMI (22.9 ±2.4 kg/m^2) participated in the total sleep deprivation protocol (Jung et al, 2011). Self-reported hunger and appetite were assessed with visual analog scales. Hunger tended to follow meal patterns during total sleep deprivation and nightshift work decreasing after food consumption. Hunger levels were lower at night, even in the absence of meals, and were similar to levels observed following meals consumed during the day. Exploratory factor analysis revealed three factors explaining 86% of the total cumulative variance, representing constructs of main course appetite, hunger, snacks/dessert appetite, and caffeine/thirst ratings. In the presence of meals, levels of the three appetite and hunger factors were lower under simulated shift work at night compared to the day. In the absence of meals during sleep deprivation, factors 1 and 3 were lower overnight and the following day during extended wakefulness and factor 2 showed a steady increase across the night. Findings suggest that hunger and appetite are unlikely to increase food intake at night.

INTRODUCTION

The obesity epidemic is a major health concern facing society. Despite advances in research and technology and increases in awareness and education, obesity in modern society continues to increase. It is predicted that by 2030 over half of the world's population will be obese (Finkelstein et al, 2012). Concurrently, ~20% of people in modern society work non-traditional hours during typical sleep time, including emergency medical staff, security personnel and members of the military (McMenamin, 2007). Working non-traditional hours has been associated with increased incidence of obesity, diabetes, and the metabolic syndrome (Markwald, 2012). This increased metabolic health risk, seen especially in shift workers, is thought to be related in part to caloric intake at adverse circadian times, yet little is known about how this altered pattern of sleep and wakefulness affects many processes regulating food intake.

Shift workers are required to be awake at abnormal times which impacts their sleep and activity patterns. Food intake is altered in shift working populations with more calories consumed in cold meals and snacks than in day workers (Waterhouse et al, 2003). Decreased availability of healthy food choices has also been shown in shift workers (Stewart and Wahlqvist, 1985; Waterhouse et al, 2003). Total sleep deprivation has been shown to increase feelings of hunger (Schmid et al, 2008; Benedict et al, 2012) whereas advancing or delaying the relationship between wakefulness and circadian timing did not change feelings of hunger (Gonnissen et al, 2012). Appetite and hunger are two contrasting constructs used to evaluate the likelihood of food intake based on two opposite factors (Blundell et al, 2010). Appetite especially refers to the qualitative aspects of food intake, including selection, motivation and preference, whereas hunger is more directly tied to physiological changes prompting food intake, including sensing changes in the feelings in the head, limbs, or stomach. These two constructs

can be likened to hedonic (appetite) versus homeostatic (hunger) influence on food intake (Lowe and Butryn, 2007). Independently and in concert they play an important role in food intake behaviors.

Hunger, appetite and food intake are also regulated by hormone secretion and signaling in the periphery as well as specific brain areas. A milieu of hormones have been implicated in the signaling of hunger and satiety throughout the body. Briefly, leptin, a satiety signal released by the adipocytes to signal sufficient energy stores in the brain (Weigle et al, 1997; Licinio et al, 2004), and ghrelin, a stomach-derived signal to increase food intake, work to balance hunger and satiety to regulate food intake behaviors as a function of energy stores (Kojima et al, 1999; Ariyasu et al, 2001). The signaling of hunger and satiety in the periphery as well as the brain is a complex process, which, interestingly, shares some overlap with centers of circadian and sleep regulation. The hypothalamus, considered the primary brain area regulating food intake behaviors (Schwartz et al, 1996b; Satoh et al, 1997) which also contains the master clock of circadian timing (suprachiasmatic nucleus, SCN), is the site of receptors in the signaling cascade for the hunger signal ghrelin, including orexigenic neuropeptide Y (NPY) and agouti-related protein (AGRP) neurons (Kamegai et al, 2001; Nakazato et al, 2001) which then in turn project to the paraventricular nucleus (PVN), important dopamine-containing reward area and the lateral hypothalamus (LH), an area involved in promotion of brain arousal (Swaab et al, 2003; Saper et al, 2005). In obesity leptin and ghrelin are dysregulated (Pelleymounter et al, 1995; Schwartz et al, 1996a; Montague et al, 1997; Farooqi et al, 2001), which could be one explanation for altered food intake behavior in this disease state. Additionally, alterations in these hormones have been demonstrated in circadian misalignment (Scheer et al, 2009; Nguyen and Wright, 2010) and insufficient sleep studies (Spiegel et al, 2004; Bass and Takahashi, 2010).

A circadian variation in hunger ratings in the presence of food has been demonstrated using the forced desynchrony protocol (Scheer et al, 2013; Sargent et al, 2016). Overall ratings of hunger were shown to peak in the early evening and to be at a minimum in the morning prior to or near habitual waketime. Further Scheer and colleagues (Scheer et al, 2013), showed hunger ratings for specific foods such as sweet, salty and starchy, foods, and fruits, meats/poultry to vary with circadian phase. However, this protocol distributes caloric intake across circadian phase and does not account for the influence of extended wakefulness, which often occurs prior to overnight shiftwork. Therefore, two protocols were used to compare appetite for a variety of foods and hunger ratings during simulated overnight shiftwork and total sleep deprivation with and without food intake.

MATERIALS AND METHODS

Subjects

A total of twenty-one healthy adults participated. Fourteen adults (n=6 males) aged (26.4 ±1.2 y), BMI (22.7 ±0.5 kg/m^2) participated in the simulated shiftwork protocol (McHill et al, 2014) and seven adults (n=5 males) aged (22 ±5 y), BMI (22.9 ±2.4 kg/m^2) participated in the total sleep deprivation protocol (Jung et al, 2011). Study procedures were approved by the Scientific Advisory and Review Committee of the Colorado Clinical and Translational Sciences Institute and by the Colorado Multiple Institutional Review Board (IRB). Subjects gave written informed consent and underwent health screening at the Sleep and Chronobiology Laboratory and the University of Colorado Boulder Clinical and Translational Research Center (CTRC). Health status of subjects was determined based on clinical history, physical exam, blood chemistries including a complete blood cell count and comprehensive metabolic panel, 12-lead

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clinical electrocardiogram and medication-free status. Subjects were low to moderate caffeine users. Detailed health and sleep and circadian rhythm disorder history status was assessed at the Sleep and Chronobiology Laboratory and a polysomnographic (PSG) sleep disorder screening night verified subjects were free of any sleep disorders. Exclusion criteria consisted of any current or chronic medical or psychiatric conditions, shiftwork or dwelling below the Denver altitude (elevation 1600 m) in the year prior, travel across more than one time zone in the 3 weeks prior to the laboratory procedures, BMI outside the normal range of 18.5 to 24.9, recent self-reported weight loss, physically active lifestyle (>1h of structured exercise per week), pregnancy, or a habitual sleep duration of <7h or >9.25h.

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 for a minimum of three days prior caffeine, alcohol, nicotine and over-the-counter medication use was proscribed. The sleep schedule was verified via 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 recorder. Drug free status was determined by self-report and verified by urine toxicology at CTRC screening and by urine toxicology and breath alcohol tester (Lifeloc Technologies Model FC10, Wheat Ridge, CO) upon UCH CTRC admission. Prior to admission, exercise was proscribed for a minimum of two days and subjects were provided a three-day outpatient diet that was designed to meet their individual daily caloric needs as determined from resting metabolic rate with a 1.5 activity factor to control energy intake. Subjects were instructed to consume meals provided and nothing else but water to ensure energy balance at the start of

CTRC protocol. Diets were prepared by the UCH CTRC Nutrition Core and contained macronutrient contents of 30% fat, 55% carbohydrate, and 15% protein in the shift work protocol and 30% fat, 50% carbohydrates, and 20% protein in the sleep deprivation protocol, and no caffeine.

Experimental Procedures

In the simulated shiftwork protocol subjects lived on the UCH CTRC for ~6 days to simulate a daytime work schedule and three consecutive night shifts (Figure 1). In the sleep deprivation protocol subjects lived on the CTRC for ~4 days that included 40h of total sleep deprivation (Figure 1). In both protocols, subjects arrived ~6-7 hours before habitual sleep time as determined from the week of 8h self-selected sleep schedules. Day 1 for both protocols included a polysomnographically (PSG)-recorded 8h sleep opportunity at the subjects' habitual bedtime and to verify that subjects were free from sleep disorders. Upon awakening at habitual time and for the duration of both protocols, subjects maintained a modified constant posture protocol; seated semi-recumbent posture in a hospital bed with the head raised to ~35 degrees, room temperature maintained 22-24º C, dim lighting (<1 lux in the angle of gaze, <5 lux maximum) during scheduled wakefulness and 0 lux during scheduled sleep. Wakefulness and subject compliance during the constant posture protocol was verified via continuous monitoring by research staff and electroencephalography (EEG) recordings.

Day 2 of the simulated shift work protocol 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 night shift. Subjects were awakened at habitual wake time and scheduled to a 2h afternoon nap opportunity prior to the nightshift. After the first night shift, subjects were

given an 8h daytime sleep opportunity that began one hour after their habitual baseline

waketime. This was followed by two more days of night shiftwork (Fig. 1). In the sleep

deprivation protocol, Day 2 also served as a baseline day of 16h scheduled wakefulness and an

8h sleep opportunity. Days 3-4 consisted of 40h of total sleep deprivation followed by an 8h

recovery sleep episode (Sleep deprivation 1-40h awake and 8h recovery sleep).

Subjects received scheduled meals (percent of daily caloric intake, 30% breakfast, 30%

lunch, 30% dinner, and 10% snack) daily. In both protocols, food served for each meal was the

same (e.g. food served at breakfast was the same across all days whether breakfast occurred

during the habitual morning or night) and subjects were instructed to consume all food provided.

In the simulated shiftwork protocol, meals were scheduled 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).

In the sleep deprivation protocol, meals were provided at approximately the same relative

time of day as the baseline Day 2 of the simulated shiftwork protocol. Thus participants were not

fed during the night of their total sleep deprivation. A single subject did not finish one dinner

during the 25-40h of total sleep deprivation, otherwise subjects consumed the diet provided.

Appetite for specific foods (dairy, meat, vegetables, fruits, sweets, bitter, salty, sour and

starchy foods), thirst, caffeine, desire to eat, fullness, preoccupation with thoughts of food,

overall hunger, and amount of food that could be eaten were assessed using visual analog scales

every 2 hours after awakening in the simulated shiftwork protocol and every hour in the sleep
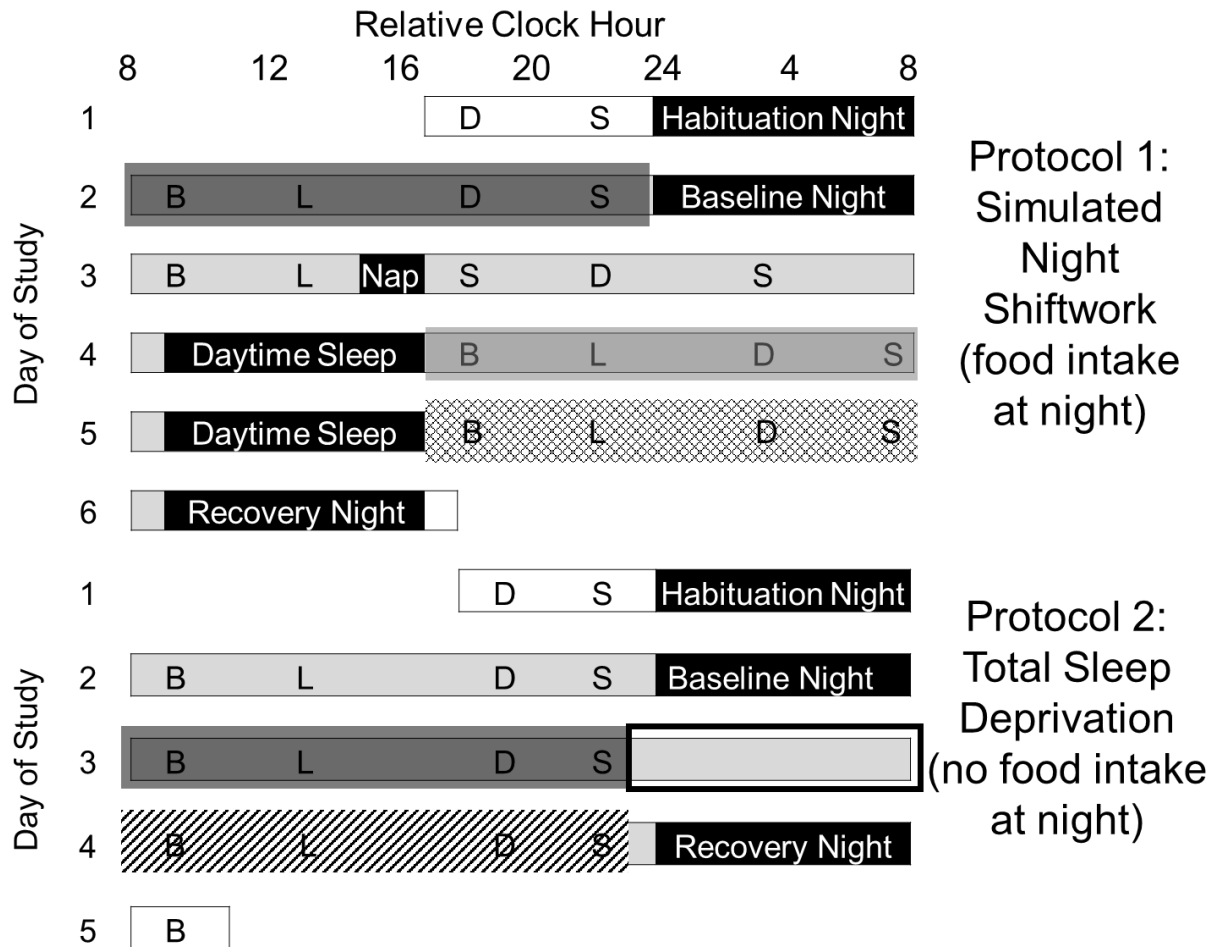
deprivation protocol.

Figure 1. Study Protocols. White bars represent room light (<40 lux), all other times of scheduled wakefulness subjects were in dim light (<8 lux, gray bars). Black bars represented scheduled sleep episodes. Both studies are shown with schedules in relative clock hour, with a baseline sleep schedule from relative clock hour 0:00-8:00, with day of study on the left y-axis. Actual sleep and wake schedules at baseline were based on subject's habitual times. All meals were scheduled throughout the protocol (B = Breakfast, L = Lunch, D = Dinner, S = Snack). All further figures will use color and patterns as are coded on protocol figures. Protocol 1 was designed to simulate night shift work with a baseline day (Study Day 1) consisting of wakefulness and scheduled meals occurring during the day (black box) and 8h of sleep opportunity scheduled overnight. Day 3 was used as a transition day to the nightshift and was not included in these analyses. Days 4 and 5 were simulated night shift work with wakefulness and meals scheduled overnight (at equivalent times after wakefulness as during day work, Day 4 in red and Day 5 in blue) and sleep scheduled during the day. Protocol 2 was total sleep deprivation with meals scheduled at consistent times after habitual waking during the day and no food intake overnight. Baseline habitual wakefulness (hours awake 1-15, black box) was compared to overnight wakefulness (hours awake 16-24, open box with black outline) and extended wakefulness on the following day (hours awake 25-39, box with diagonal lined pattern).

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Statistical Analyses

Factor analysis was used to describe the structure of variables in terms of a smaller number of underlying dimensions using the analysis of shared variance based upon factor loadings on baseline day. Factor analysis was completed using baseline responses combined for both protocols, measured every 2h for the 16h of wakefulness on Day 2 in Protocol 1 – simulated night shiftwork and for the first 16h of Protocol 2 – total sleep deprivation. Orthogonal (Varimax) rotation was used and factors were determined by eigenvalues greater than one. Data in text are presented as mean ± SEM.

Factors were analyzed every two hours (Protocol 1 – simulated night shiftwork) or hourly (Protocol 2 – Total Sleep Deprivation) and averaged for the simulated work shift in Protocol 1 (hours awake 2-14) and for habitual daytime (hours awake 1-15 and 25-39) and habitual nighttime (hours awake 16-24) in Protocol 2. Sex was initially included in statistical models to examine possible effects of sex but results were not significant thus it was removed for final analyses. For Protocol 1, data were analyzed using mixed model ANOVA with work shift or hours awake as fixed factors, and subject as a random factor using STATISTICA version 13 (StatSoft). Two-tailed dependent t test planned comparisons were used to compare group averaged hunger and appetite scores at each time point between scheduled day shift and night work shifts 2 and 3 and for work shift averages. For Protocol 2, data were also analyzed using mixed model ANOVA with hours awake or time bin of hours awake as fixed factors and subject as a random factor. Two-tailed dependent t test planned comparisons were also used to compare two-hourly responses between day and night work in Protocol 1, as well as group average hunger and appetite scores between habitual daytime, nighttime and subsequent daytime. One subject

was determined to be an extreme outlier as values were greater than 3 times the interquartile range for Factor 3 and therefore was removed from statistical analyses of that factor.

RESULTS

Exploratory factor analysis found four factors that accounted for 86% of the total cumulative variance at baseline. Factor 1 consisted of how strong is your desire to eat dairy, bitter, meat, sour, and starchy foods (Figure 2). This factor grouped on the construct of main course appetite or the psychological drive or desire to eat specific foods often found in the main meal course. Factor 2 consisted of questions about hunger including how strong is your desire to eat, how full are you, how preoccupied are you with thoughts of food, how hungry are you, and how much could you eat right now (Figure 3). This factor largely grouped on the construct of hunger or the general drive to intake food to fulfill a physiological need. Factor 3 consisted of how strong is your desire to eat fruits, sweets and salty foods (Figure 4). This factor grouped on the construct of snacks/dessert appetite or the psychological drive or desire to eat specific foods often snacks or desserts. Factor 4 consisted of how strong is your desire to consume caffeine and how thirsty are you. This factor grouped on the constructs of caffeine and thirst. The only additional item that did not significantly load on one of the factors was how strong is your desire to eat vegetables.

Factor 1 (main course appetite) was lower during many hours awake of both night shifts compared to the day shift in Protocol 1 – simulated night shiftwork (time by day interaction, $p=0.057$, main effect of time and day, $p<0.005$; Figure 2). Specifically, there were no differences in main course appetite ratings between day and night shift work at 2 hours awake, however at all subsequent time points appetite ratings on one or both of the night shifts were significantly lower than on the baseline day shift (all $p<0.05$; Figure 2A). Additionally, main course appetite

ratings were lower on average for both night shifts compared to the day shift (both p<0.05;

Figure 2B). Main course appetite ratings followed meal patterns during the day in Protocol 2 –

total sleep deprivation but remained low across the night without food intake (main effect of

time, p<0.00001; Figure 2C) Further, main course appetite ratings were significantly lower

during overnight wakefulness (hours awake 16-24), even without food intake as well as during

extended wakefulness the following day (hours awake 25-39) with food intake at identical clock

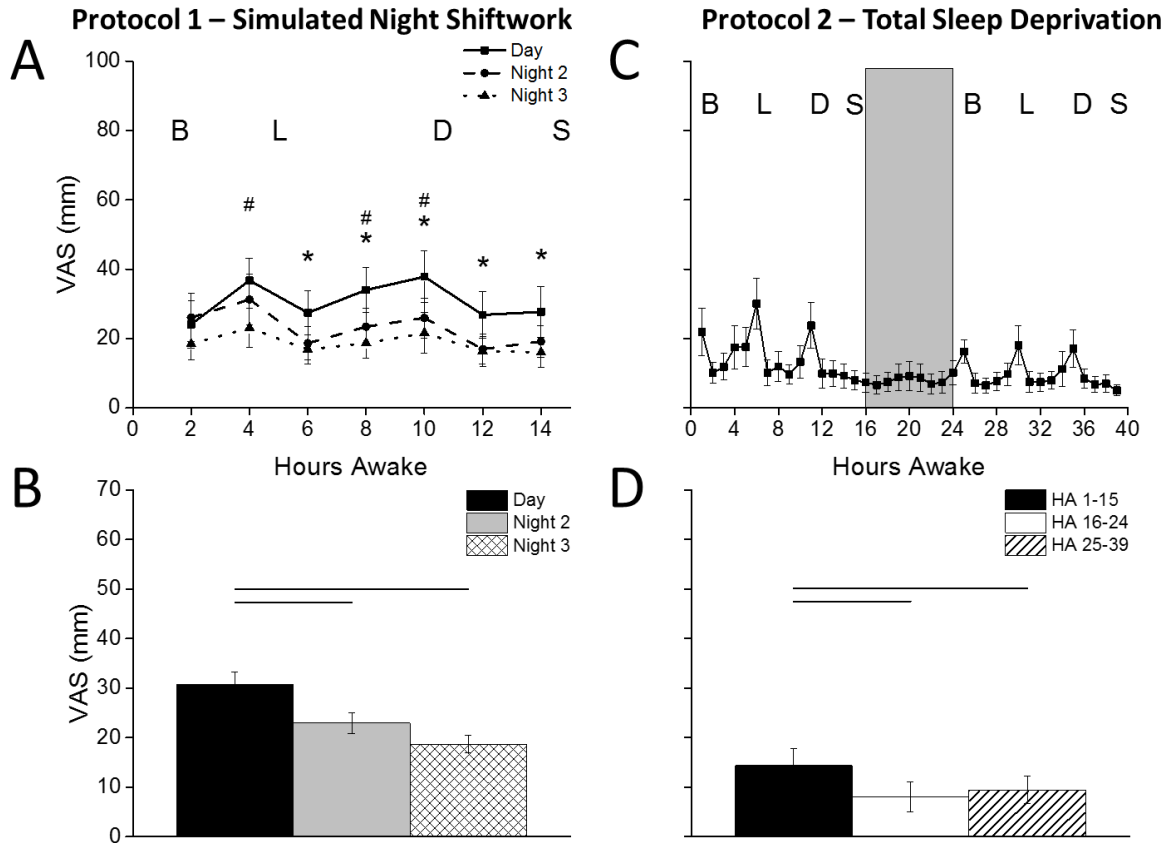hours as during baseline (hours awake 1-15) (both p<0.05; Figure 2D).

Figure 2. Factor 1 – Main Course Appetite. Factor 1 (Main Course Appetite) was measured during simulated night shiftwork (A and B) and total sleep deprivation (C and D). Meals are represented on panels A and C as in Figure 1, B = Breakfast, L = Lunch, D = Dinner, S = Snack. Significant hourly differences are represented as follows: * = Day 2 vs Day 4, # = Day 2 vs Day 5; $ = Day 4 vs Day 5. Significant daily averages are represented by a black line between the two days or time bins being compared. Main course appetite scores in Protocol 1 – simulated night shiftwork were collected every two hours on baseline day work (A – black line; B – black bar), and after the second (A – red line; B – red bar) and third (A – blue line; B – blue bar) days of night shift work. Main course appetite scores in Protocol 2 – total sleep deprivation were collected hourly (C) across baseline habitual wakefulness with habitual scheduled meals (hours awake 1-15, D – black box), overnight wakefulness without food intake (hours awake 16-24, D – open box with black outline), and extended wakefulness the following day with habitually scheduled meals (hours awake 25-39, D – box with diagonal lined pattern).

Factor 2 (hunger) was significantly lower during most hours awake of both night shifts compared to the day shift in Protocol 1 – simulated night shiftwork (time by day interaction, $p<0.05$; Figure 3). There were no differences in hunger ratings between day and night shiftwork within the first 4 hours after awakening (after breakfast and prior to lunch), however at all subsequent time points hunger ratings on one or both of the night shifts were significantly lower than on the baseline day shift (all $p<0.05$; Figure 3A). Hunger ratings at 8h after awakening were also lower on night shift 3 than night shift 2 ($p<0.05$). Additionally, hunger ratings were lower on average for both night shifts compared to the day shift (both $p<0.005$; Figure 3B). Hunger ratings in Protocol 2 – total sleep deprivation increased before and decreased after meals during the day, and increased at a steadily overnight without meals (main effect of time, $p<0.00001$; Figure 3C). Further, average hunger ratings were similar during overnight wakefulness (hours awake 16-24) compared to baseline daytime wakefulness (hours 1-15; $p=0.95$), and there was a trend for hunger to be decreased during extended wakefulness the following day (hours awake 25-39; Figure 3D) compared to baseline daytime (hours awake 1-15; $p=0.06$) and overnight wakefulness (hours awake 16-24; $p=0.05$).
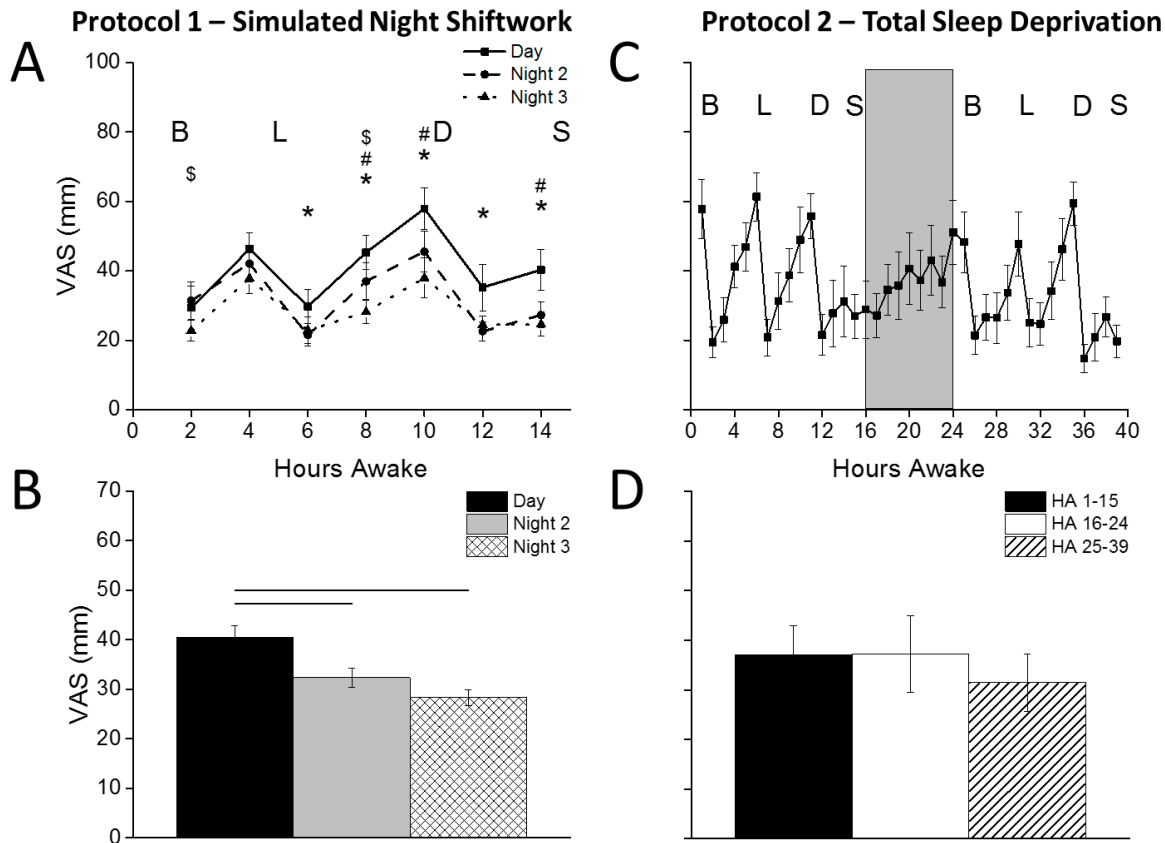
Figure 3. Factor 2 – Hunger. Factor 2 (hunger) was measured during simulated night shiftwork (A and B) and total sleep deprivation (C and D). Meals are represented on panels A and C as in Figure 1, B = Breakfast, L = Lunch, D = Dinner, S = Snack. Significant hourly differences are represented as follows: * = Day 2 vs Day 4, # = Day 2 vs Day 5; $ = Day 4 vs Day 5. Significant daily averages are represented by a black line between the two days being compared. Hunger scores in Protocol 1 – simulated night shiftwork were collected every two hours on baseline day work (A – black line; B – black bar), and after the second (A – red line; B – red bar) and third (A – blue line; B – blue bar) days of night shiftwork. Hunger scores in Protocol 2 – total sleep deprivation were collected hourly (C) across baseline habitual wakefulness with habitual scheduled meals (hours awake 1-15, D – black box), overnight wakefulness without food intake (hours awake 16-24, D –open box with black outline), and extended wakefulness the following day with habitually scheduled meals (hours awake 25-39, D – box with diagonal lined pattern).

Factor 3 (snacks/dessert appetite) was significantly lower at many hours awake of both night shifts compared to day shift in Protocol 1 – simulated night shiftwork (time by day interaction, $p < 0.0005$; Figure 4). There were no significant differences in snacks/dessert appetite ratings between day and night shiftwork at 2h (following breakfast) and 6h (following lunch) after awakening, however at all subsequent time points snacks/dessert appetite ratings on one or both of the night shifts were significantly lower than on the baseline day shift (all $p < 0.05$; Figure 4A). Snacks/dessert appetite ratings at 8h after awakening were also lower on night shift 3 than night shift 2 ($p < 0.05$). Additionally, snacks/dessert appetite ratings were lower on average for both night shifts compared to the day shift (both $p < 0.01$; Figure 4B). Snacks/dessert appetite ratings for Protocol 2 – total sleep deprivation generally increased before and decreased after meals and were low overnight (main effect of time, $p < 0.000001$; Figure 4C). Further, average snacks/dessert appetite ratings were lower during overnight wakefulness (hours awake 16-24, $p < 0.05$) and extended wakefulness the following day (hours awake 25-39, $p < 0.05$) compared to baseline (hours awake 1-15; Figure 4D).
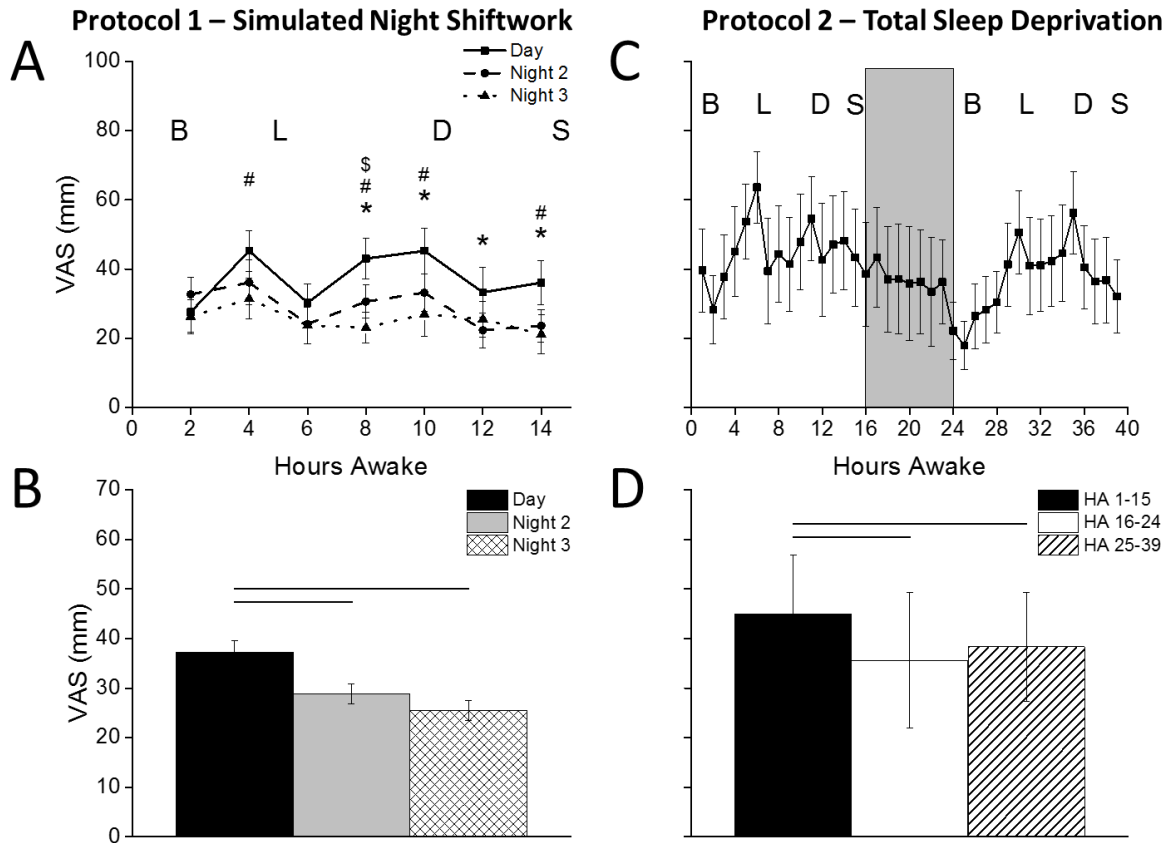
Figure 4. Factor 3 – Snacks/Dessert Appetite. Factor 3 (snacks/dessert appetite) was measured during simulated night shiftwork (A and B) and total sleep deprivation (C and D). Meals are represented on panels A and C as in Figure 1, B = Breakfast, L = Lunch, D = Dinner, S = Snack. Significant hourly differences are represented as follows: * = Day 2 vs Day 4, # = Day 2 vs Day 5; $ = Day 4 vs Day 5. Significant daily averages are represented by a black line between the two days being compared. Appetite scores in Protocol 1 – simulated night shiftwork were collected every two hours on baseline day work (A – black line; B – black bar), and after the second (A – red line; B – red bar) and third (A – blue line; B – blue bar) days of night shiftwork. Appetite scores in Protocol 2 – total sleep deprivation were collected hourly (C) across baseline habitual wakefulness with habitual scheduled meals (hours awake 1-15, D – black box), overnight wakefulness without food intake (hours awake 16-24, D –open box with black outline), and extended wakefulness the following day with habitually scheduled meals (hours awake 25-39, D – box with diagonal lined pattern).

Factor 4 (caffeine/thirst) showed a significant decrease across time in the shift (main effect of time; $p<0.01$) but was not different between day and night shifts at any time point in the first 8h on the work shift. Between 10 and 16 hours after awakening, caffeine/thirst ratings on one or both of the night shifts were significantly lower than on the baseline day shift (all $p<0.05$; Figure 5A). Additionally on average between days in Protocol 1, there was a trend for lower caffeine/thirst ratings between day and night shift 2 ($p=0.074$) and night shift 3 ($p-0.057$; Figure 5B). Caffeine/thirst ratings remained low throughout Protocol 2 – total sleep deprivation and was not statistically different on average between overnight wakefulness (hours awake 16-24), extended wakefulness the following day (hours awake 25-39) and baseline wakefulness (hours awake (1-15), (all $p>0.21$). There was a trend for increased caffeine/thirst ratings during overnight wakefulness (hours awake 16-24) compared to extended wakefulness the following day (hours awake 25-39, $p=0.084$; Figure 5C and 5D).
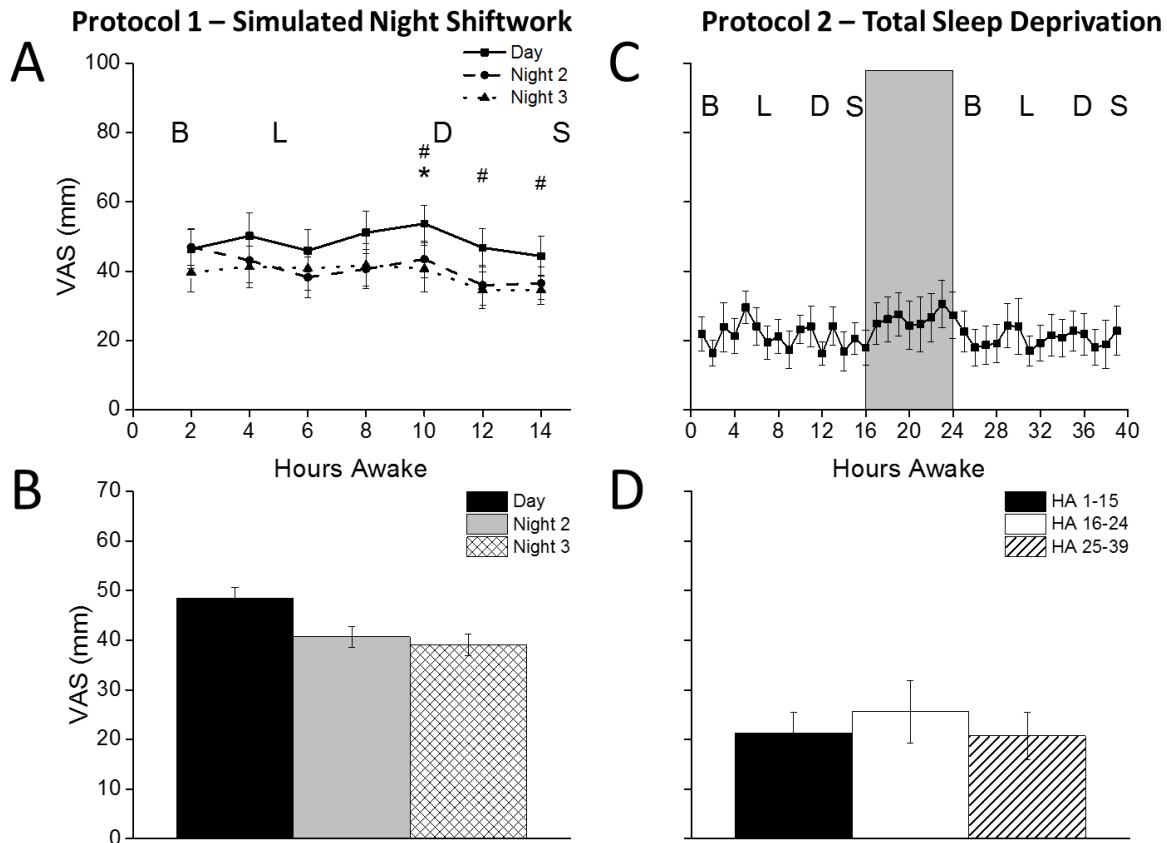
Figure 5. Factor 4 – Caffeine/Thirst. Factor 4 (caffeine/thirst) as measured during simulated night shiftwork (A and B) and total sleep deprivation (C and D). Meals are represented on panels A and C as in Figure 1, B = Breakfast, L = Lunch, D = Dinner, S = Snack. Significant hourly differences are represented as follows: * = Day 2 vs Day 4, # = Day 2 vs Day 5; $ = Day 4 vs Day 5. Significant daily averages are represented by a black line between the two days being compared. Caffeine/thirst scores in Protocol 1 – simulated night shiftwork were collected every two hours on baseline day work (A – black line; B – black bar), and after the second (A – red line; B – red bar) and third (A – blue line; B – blue bar) days of night shift work. Caffeine/thirst scores in Protocol 2 – total sleep deprivation were collected hourly (C) across baseline habitual wakefulness with habitual scheduled meals (hours awake 1-15, D – black box), overnight wakefulness without food intake (hours awake 16-24, D –clear box with black outline), and extended wakefulness the following day with habitually scheduled meals (hours awake 25-39, D – box with diagonal lined pattern).

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DISCUSSION

Ratings of appetite for specific foods, including main course appetite and snacks/dessert appetite as well as general hunger and caffeine/thirst ratings were found to change with circadian misalignment and sleep deprivation. During circadian misalignment in simulated night shiftwork, main course appetite, snacks/dessert appetite and hunger were lower during the night shifts than during the day. Caffeine/thirst ratings showed a trend for the same decrease during night shifts compared to day. During sleep deprivation, main course appetite, snacks/dessert appetite and hunger generally followed meal patterns during the day with an increase before and a decrease after meals. During overnight fasting of sleep deprivation, both types of appetite were lower whereas hunger steadily increased when no meals occurred. Caffeine/thirst ratings were low during total sleep deprivation. Our findings demonstrate that both simulated night shift and total sleep deprivation protocols impact hunger and appetite ratings.

A circadian rhythm in overall hunger and appetite has been demonstrated in two previous studies with a trough in the biological morning and a peak in the biological evening. In one study the trough corresponded to ~8AM and the peak to ~8PM (Scheer et al, 2013) and this pattern was consistent for overall hunger as well as for specific foods including, sweets, salty and starch foods, fruits, and meats/poultry. In the other study, the trough occurred between 0100-0500 and the peak between 1700-2100 (Sargent et al, 2016). Our findings suggest that hunger follows meal patterns throughout the day during both day and night shifts, with the highest hunger ratings in Protocol 1 – simulated shift work occurring prior to dinner which occurred during the overnight hours on night shifts. Decreased ratings during night shifts may support previous findings, as the majority of meals occurred following the peak hunger time, and were found to be decreased on average compared to day shift when meals were occurring during the time following the trough

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of hunger an leading to the peak. In Protocol 2 – total sleep deprivation, hunger increased to similar levels immediately prior to each meal during the daytime hours after but decreased rapidly after food intake and remained low overnight. Additionally, hunger increased consistently overnight but did not achieve the same maximal hunger levels seen during the previous day, despite a >8 hour overnight fast. Our finding that hunger ratings increased overnight without food intake but remained lower than peak daytime levels despite an extended fast, are consistent with the notion that the circadian system plays a role in regulating overnight hunger. Appetite for main course and snacks/dessert showed the same pattern of increased values prior to meals in both protocols and both were reduced during night shiftwork with food intake and overnight wakefulness without food intake. This is consistent with previous research suggesting a circadian influence on appetite for certain foods that are included in these factors. Together, these findings suggest that the circadian system may influence hunger and appetite to remain at lower levels than during daytime wakefulness to assist with the regulation of the timing of food intake to proper circadian times when other necessary physiological processes (i.e. digestion, metabolism) are prepared for food intake.

Many factors contribute to food intake in addition to hunger, including appetitive hormonal changes and food availability. Here, we examined simulated night shiftwork with circadian misalignment compared to sleep deprivation. In previously published data from Protocol 1, leptin and PYY were found to be lower on night shift 2 compared to baseline and no change in ghrelin was found (McHill et al, 2014). Our findings show hunger was lower on all night shifts, which occurs despite no change in the hunger hormone ghrelin and lower levels of the satiety hormone leptin. This suggests that the circadian system may play a role in regulating hunger separately from the hormonal regulation of hunger. Findings from field studies have

shown that both leptin and ghrelin are decreased in actual shift workers compared to day workers (Crispim et al, 2011), who are exposed to circadian misalignment, which may suggest an influence of the circadian system. Others have found that circadian misalignment induced by repeated exposure to shortened daylength, showed no changes in levels of ghrelin or leptin but that their secretion patterns shifted to respond to shift in meal patterns as opposed to the circadian clock (Gonnissen et al, 2012). This is in contrast to the current findings and other studies of total sleep deprivation, which find increased morning plasma ghrelin and hunger (Schmid et al, 2008), as well as increases in hunger without changes in overall food intake (Benedict et al, 2011). Together these results suggest that sleep and circadian timing may interact to impact hunger and satiety hormones but that may not translate to subjective hunger ratings or actual food intake independent of other influences, such as food intake.

Food availability is also an important factor in food intake. Being awake at night, by definition, increases the daily length of the opportunity to eat assuming that food is readily available as in modern society. Sleep restriction studies have demonstrated that increased food intake occurs in the evening when food is available (Nedeltcheva et al, 2009; Markwald et al, 2013; Spaeth et al, 2013). Sleep restriction has been shown to increase food intake when food is available *ad libitum* and this is associated with increased leptin and decreased ghrelin and hunger ratings (Markwald et al, 2013). This suggests that food availability can play a stronger role in food intake behaviors in comparison to physiology. However, another study found during the morning after total sleep deprivation, ghrelin and subjective hunger were both increased compared to baseline sleep and larger portion sizes of foods were chosen (Hogenkamp et al, 2013). This study is consistent with the current findings, showing that hunger increases in the morning and suggests that this can influence food intake behaviors. Recently, focus on the

circadian system has been increasing as it has been shown to influence metabolism and increase weight gain such that eating at an adverse circadian time promotes a state of weight gain (Arble et al, 2009; McHill et al, 2014). The current findings demonstrate that appetite and hunger are decreased overnight, suggesting that if food intake occurs it is not necessarily as a result of appetite or hunger. Potentially, this food intake is due instead to food availability, or the perception that meals are to be consumed at regular intervals and if someone is awake that they should be eating, though they may not necessarily be driven by their physiology.

Shift workers and many others in our modern society keep abnormal sleep and wakefulness patterns due to their work schedules and domestic and social pressures. In addition to directly impacting food behaviors and metabolism, circadian misalignment and sleep deprivation have been shown to impact other relevant physiological processes, including stress and activity patterns. A limitation of our studies is that they were both performed in controlled laboratory settings, with controlled meals, physical activity, and lighting, which do not replicate the potential physical demands or stress incurred during shift work or sleep deprivation. Another limitation of these studies is that hunger was only assessed using subjective visual analog scale. Future studies should incorporate objective measures of food intake (e.g., ad libitum meal conditions, buffet) or other assessments of the sensory components of food intake such as image/examples or smells/tastes.

There are many discrepancies in research literature between food intake behavior during sleep restriction and circadian misalignment. It is important to consider that not only hunger but also appetite for specific foods may play a role in this discrepancy. Very little is known about how the circadian system, the homeostatic drive for feeding, and signaling hormones interact during circadian misalignment to modulate hunger. The extent to which the consumption or

availability of food is impacted by hunger or how these food choices themselves impact hunger has received relatively little research attention but is nonetheless may be an important component of the occurrence of weight gain and subsequent obesity. These incongruous results suggest that more research is needed to tease apart the impact of sleep restriction and circadian misalignment on behaviors and biological processes involved in food intake and metabolism in laboratory-simulated and actual shift work environments.

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CHAPTER 4

INFLUENCE OF CHRONIC CIRCADIAN MISALIGNMENT ON

GROWTH HORMONE SECRETION

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ABSTRACT

Dysregulation of circadian and sleep processes has been shown to alter metabolic function. Growth hormone (GH) is an important metabolic hormone that is released in a pulsatile fashion throughout the day, primarily during non-rapid eye movement (NREM) slow wave sleep (SWS). Sleep disruption impacts GH secretion but relatively little work has examined the impact of chronic circadian misalignment on GH. Therefore, we tested participants using 55-day long in-laboratory protocols to examine the impact of circadian entrainment or misalignment on GH secretion. 17 healthy adults (3 female), aged 31.7 ± 6.y (±SD) participated. On ~days 5-6 of a 6-day baseline schedule with 8h sleep 16h wakefulness schedules, blood samples were drawn for plasma GH every 10 min for 32h. Next subjects were assigned to either a 24.0h or 24.6h sleep/wakefulness schedule. After ~3 weeks on these schedules blood was again sampled for GH. During these sampling windows melatonin was assessed to determine baseline circadian timing as well as entrainment or failure to entrain to the assigned day length, and sleep architecture was assessed via polysomnography. The pattern of GH secretion was altered during circadian misalignment such that secretion during the first half of scheduled sleep was significantly lower after exposure to weeks of circadian misalignment compared to baseline, despite similar duration of SWS. Further, 24h total GH secretion was preserved during chronic circadian misalignment and was similar to 24h secretion during circadian entrainment. The current findings demonstrate that chronic circadian misalignment alters the temporal pattern of GH and reduced the sleep-related GH surge.

INTRODUCTION

Many physiological processes are controlled by an intrinsic biological, or circadian, clock which keeps consistent ~24h timing of these processes through means of a molecular feedback loop (40). Sleep and wakefulness behaviors are also influenced by the circadian system. Wakefulness can occur at the wrong biological time, creating a mismatch between circadian timing and sleep and wakefulness behaviors which is referred to as circadian misalignment (1, 17, 64). This mismatch can occur chronically as in circadian rhythm sleep-wake disorders (42), acutely as in total sleep deprivation (13, 26, 29), as well as intermittently as in jet lag and shift work (41, 60, 66). Circadian misalignment has been shown to negatively impact endocrine (47, 49), cardiovascular (45, 57), immune (9, 12, 18, 65) and cognitive (28, 64) health and function. The physiological impact of circadian misalignment has been studied in the context of dysregulation of many behaviors, including sleep.

Growth hormone (GH) is secreted in a pulsatile fashion and is generally considered to be a sleep-induced hormone as it is secreted in highest concentrations during non-rapid eye movement (NREM) slow wave sleep (SWS), also referred to as stages 3 and 4 or N3 sleep (2, 16, 21, 24, 43). Sleeping during the day does not appear to change GH secretion rates or levels (39, 44, 56), whereas total sleep deprivation has been shown to reduce secretion during overnight wakefulness compared to overnight sleep (7, 8, 51), while conserving 24h total secretion (3). Recovery sleep following total sleep deprivation has been shown to increase both slow wave sleep and GH secretion area under the curve (AUC) (54). In some (46), but not all (33) studies, sleep restriction has been shown to increase GH secretion prior to sleep onset. During temporal isolation conditions, peak GH secretion was decreased during the "free-running" period compared to baseline but average secretion remained unchanged. These alterations were found

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concurrent with changes in SWS (31, 58), indicating there is a relationship between sleep and GH.

GH is primarily an anabolic hormone, promoting protein synthesis (6). GH also plays a role in regulating substrate utilization, shifting energy use from glucose to favor lipolysis (32) and decreasing glucose uptake by muscle tissue (67) as well as insulin sensitivity (10). The circadian system is also strongly involved in energy metabolism and a circadian trough in GH levels was found during typical sleep time in the biological night (50). However, a relatively small amount of research has focused on the role of the circadian system in GH secretion. Therefore, we employed a chronic circadian misalignment protocol to examine how weeks of entrainment versus misalignment between circadian timing and scheduled sleep episodes due to failure to entrain impacts GH secretion.

METHODS

The following planned analyses were part of a larger protocol that examined the impact of circadian misalignment on sleep, hormones, immune cytokines, and cognition. Detailed results and methods for circadian phase, sleep, leptin, inflammatory markers, and cognitive performance have been published elsewhere (34, 61-64).

Subjects

Samples were analyzed from a total of sixteen healthy adults (3 female), aged 31.5 ± 6.1y. Written, informed consent was obtained from subjects and procedures were approved by the Brigham and Women's Hospital Institutional Human Research Committee and data analyses were approved by the University of Colorado Boulder IRB. Subjects were deemed healthy based

on screening including medical history, physical exam, electrocardiogram, blood and urine chemistries, and urine toxicology to confirm drug-free status. Subjects also completed psychological tests and a semi-structured interview with a clinical psychologist. No subjects reported working night or rotating shifts within the prior 3 years or traveling across more than one time zone in the prior 3 months. For 3 weeks prior to laboratory admission subjects were required to keep a consistent 8-h sleep schedule at their habitual times, which was verified by concordance of sleep logs and phone call-ins to a time-stamped voice recorder. Additionally, ambulatory recordings of wrist actigraphy were used to verify compliance with sleep and wakefulness schedules for at least the final week, immediately prior to entering the laboratory. Urine toxicology screen was performed upon admission to verify subjects were drug free.

In-laboratory Conditions

Subjects lived in a private research suite for the duration of the protocol in an environment free of time cues (i.e. no watches, cell phones, computers, internet or other personal electronic devices or contacts which could provide time cue information). Ambient light, temperature, sleep and wakefulness opportunities, activity and nutrition intake (breakfast, lunch, dinner and snack; 150 mEq Na^{++}, 100 mEq K^+ ± 20%, 1500 to 2500 cc fluids, isocaloric) were strictly controlled during the study. Exercise and napping were proscribed and adherence was verified via EEG monitoring and interaction with research staff.

Testing procedures were scheduled based on habitual sleep and wakefulness timing during the ambulatory monitoring week prior to laboratory entry by subtracting 4 hours from the average midpoint of the sleep episodes from the week prior to determine bedtime. Upon entering the laboratory, subjects completed an initial habituation condition of 6 days with scheduled

procedures including meals, blood sampling, and 8-h sleep opportunities overnight (Figure 1).

Subjects were exposed to bright room light (~450 lux in the angle of gaze) during scheduled

wakefulness to ensure stable entrainment to the scheduled 24.0-h day. Blood was sampled on

~Day 5 and 6 to assess baseline GH levels and secretion. This was followed by an initial 40-h

constant routine protocol (CR) (11), to assess circadian timing by melatonin sampling under

controlled conditions, i.e. maintaining wakefulness and constant conditions, including seated,

semi-reclined posture in hospital bed with head raised to ~35º, thermoneutral temperature, dim

light (~1.5 lux in the angle of gaze), small hourly isocaloric snacks and fluid intake (caloric

content increased proportionally across snacks to account for increased energy cost of sustained

wakefulness). Following the constant routine protocol subjects were scheduled to either 24.0-h or

24.6h- days for ~25 days. In the 24.6-h day condition, scheduled sleep opportunities were

extended by 0.2-h (12 min) and wakefulness by 0.4-h (24 min), maintaining a 2:1 wakefulness:

sleep ratio as in the scheduled 24.0-h day. Blood was sampled near the end of this chronic

circadian entrainment or misalignment exposure (~Day 27) to assess the impact of chronic

circadian entrainment versus chronic misalignment on GH concentration and secretion. A repeat

constant routine protocol was then performed to reassess circadian timing from melatonin and

determine entrainment or misalignment to the scheduled daylength (63, 64). Women began

studies during menses so CRs would be during follicular phase to control for influence of
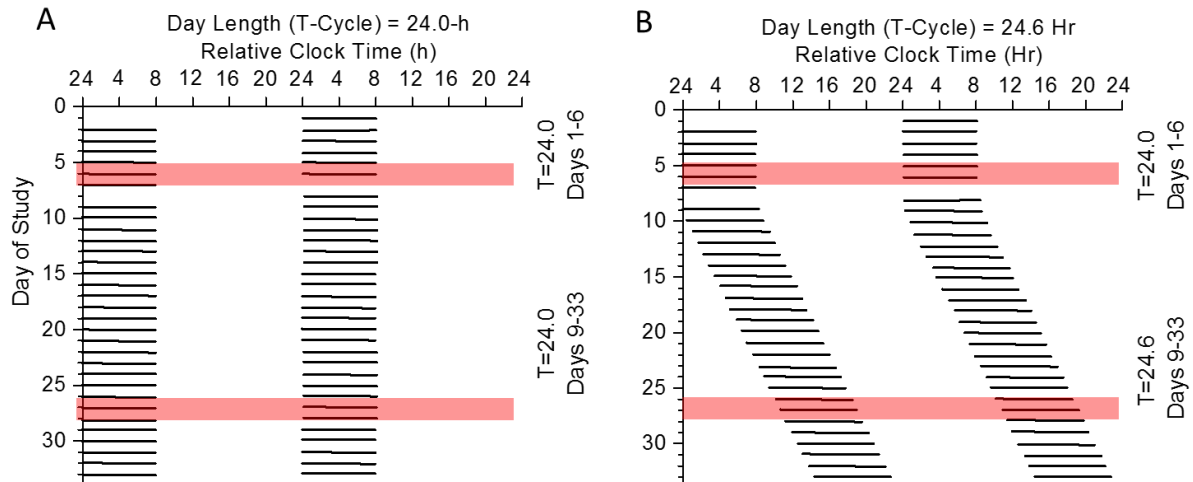
menstrual cycle on circadian phase (59).

Figure 1. Example Protocols. Protocol figures for 24.0h scheduled daylength (A) and 24.6h scheduled daylength (B). Relative clock hour (top x axis) is based on a subject with a habitual sleep time of 00:00 and wake time of 08:00. Black lines represent scheduled sleep episodes. Red lines represent periods of melatonin and growth hormone sampling.

Blood Sampling for Melatonin and Growth Hormone

Blood was sampled through an indwelling intravenous catheter throughout each scheduled blood sampling window. Patency was maintained by infusing heparinized saline (0.45% sodium chloride, 10 U of heparin/mL) at a rate of 5-10 mL/h between samples. All samples were processed immediately after collection, spun in a refrigerated centrifuge and then immediately stored at -80ºC until assayed.

Plasma samples were collected every 10 min for assessment of concentration of growth hormone (GH) assayed by chemiluminescence. GH secretory rates estimated using a waveform-independent deconvolution procedure modification of the pulse detection algorithm ULTRA (52). The deconvolution procedure allows the calculation of hormone secretory rates from plasma concentrations using a mathematical model that removes the effects of hormonal distribution and degradation. Thus, this procedure provides a more accurate estimation of the secretory process than peripheral concentrations.

Plasma samples were also collected every 30-60 minutes for assessment of melatonin levels using I^{125} radioimmunoassay (RIA). Circadian entrainment or misalignment melatonin data and analyses have been published elsewhere (34, 63, 64). Melatonin levels from the sampling windows corresponding to GH analyses are presented here for descriptive purposes.

Sleep

Sleep parameters have been previously reported in detail for this protocol with a larger sample size (34, 64), thus sleep outcomes are provided primarily for descriptive purposes.

Statistical Analyses

Changes in GH secretion were examined with mixed model ANOVA with group, sample time, sampling window, sleep/wakefulness episode, 4h bin, and/or total 24h secretion as fixed factors (Statistica, StatSoft). Planned comparisons were examined with dependent t-tests within groups for analysis of individual time points or binned data.

RESULTS

Effects of Circadian Misalignment on Sleep and Circadian Outcomes

Circadian entrainment was classified based on melatonin onset timing ($DLMO_{25\%}$) occurring consistently near bedtime across the 25-day chronic entrainment versus misalignment portion of the protocol (34, 61-64). As reported elsewhere, exposure to weeks of circadian misalignment impacted circadian timing outcomes (61-64) and sleep (34, 61, 64). Eight participants (1 female, 7 males) were classified as entrained by their circadian melatonin timing being synchronized with their respective scheduled day length. Eight participants (2 females, 6 males) were classified as misaligned due to an abnormal temporal relationship between their circadian melatonin timing and respective scheduled day length, including the timing of sleep and wakefulness opportunities. Plasma melatonin levels were low during the daytime sampling of GH and rose consistently prior to sleep in the entrained group, whereas levels were rose and fell across the daytime hours in the misaligned group, indicating that scheduled sleep did not consistently occur during the biological night (Figure 2). As reported previously, total sleep timing was decreased by ~40 min compared to baseline in the misaligned group when scheduled sleep occurred out of phase with the biological night (34). Furthermore, a significant increase in wakefulness after sleep onset (WASO) (48.9 min) with a concurrent decrease in Stage 2 (34.6

min), and sleep efficiency (8%) was observed after weeks of circadian misalignment compared to baseline. Interestingly, there was no significant difference in amount of slow wave sleep (SWS) for either group when comparing baseline to either entrained or misaligned sleep episodes.
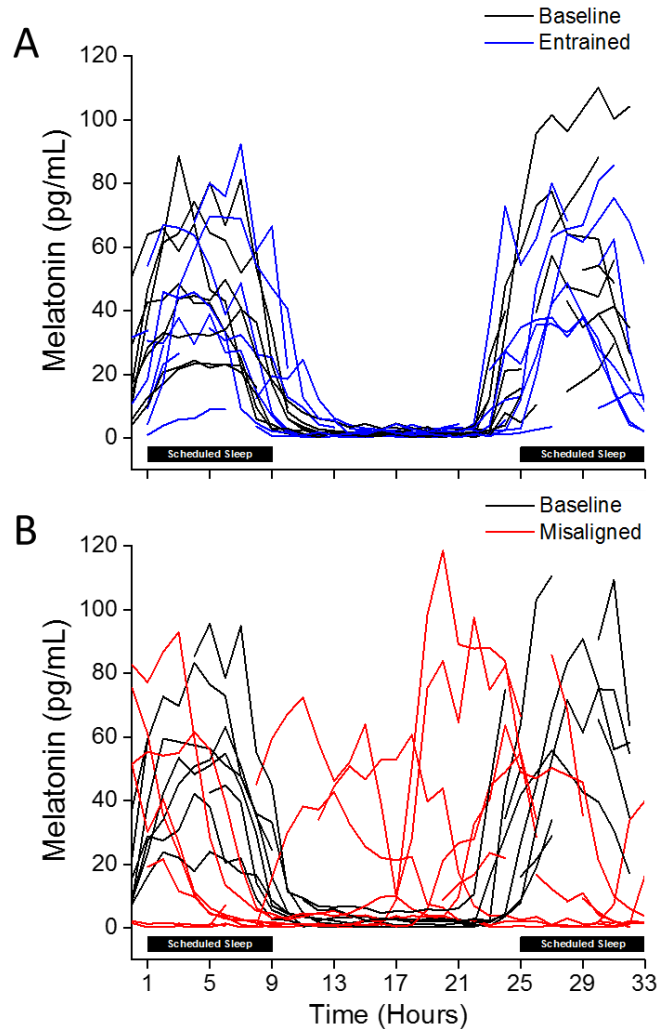
Figure 2. Melatonin Levels. Melatonin levels are shown for individual subjects across the first and second 36h sampling periods, at baseline after exposure to weeks of circadian entrainment versus misalignment for both the entrained (A) and misaligned (B) groups. Each line represents one subject, with subjects classified as entrained represented by a black line at baseline and solid blue line at the second sampling window (A) and misaligned as a black line at baseline and a solid red line at the second sampling window (B). Scheduled sleep is denoted by black bars at the bottom of each graph. Error bars represent ± SEM.

Effects of Circadian Misalignment on GH Secretion

Growth hormone secretion was altered by misalignment between circadian timing and scheduled sleep and wakefulness behaviors (Figure 3). A main effect of time was found for both entrained and misaligned groups and an interaction of time and sampling window was found in the misaligned group when analyzed separately ($p < 0.00001$). Peak GH secretion occurred during the first half of sleep episodes regardless of group or sampling window. Total GH secretion for sleep and wakefulness segments was similar to baseline in the entrained group, but was decreased during both sleep episodes when compared to baseline during chronic circadian misalignment (Figure 4). Analysis of 4h bins were used to examine changes in GH secretion in early vs late sleep and other times of day. GH secretion levels were similar in time bins of sleep and wakefulness in the circadian entrainment group compared to baseline (Figure 5). However, GH secretion was significantly decreased in the first 4h of each scheduled sleep episode in the circadian misalignment group compared to baseline (Figure 5). Total 24h GH secretion was found to be similar across the study within group (Figure 6).
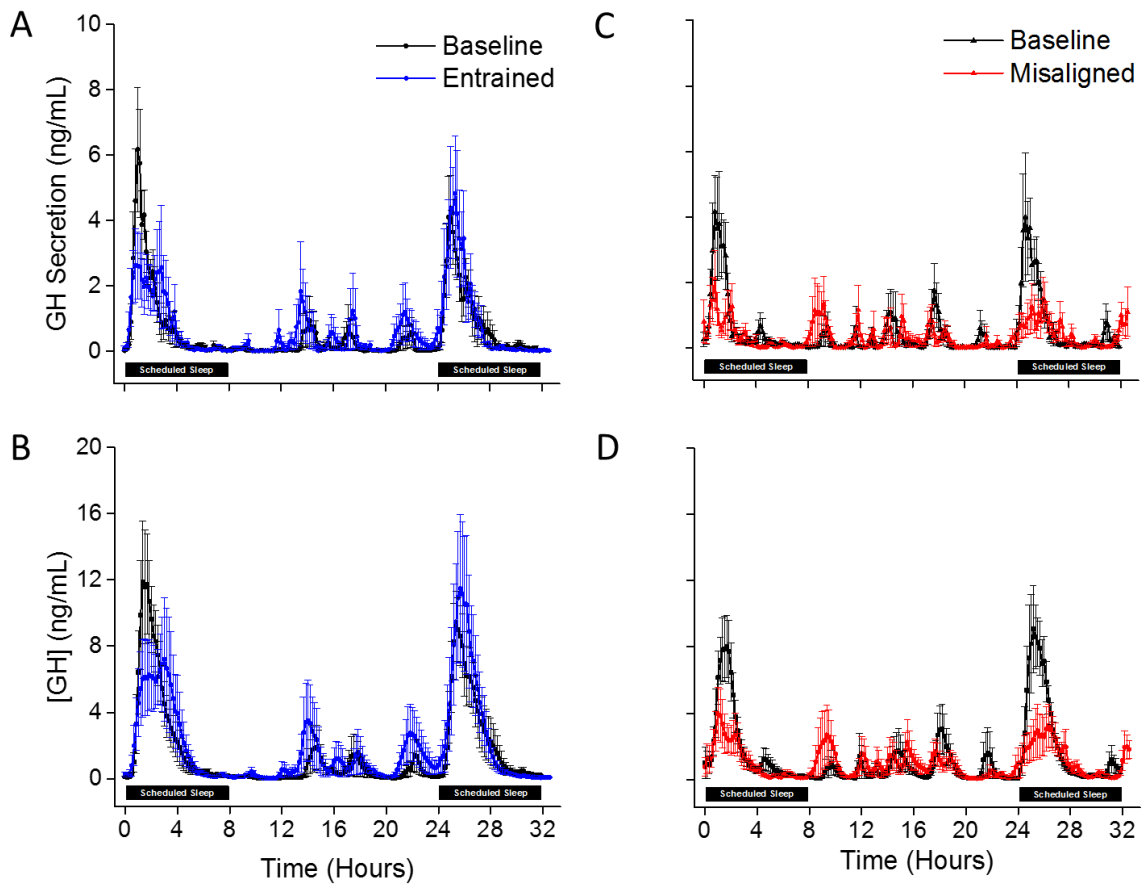
Figure 3. Growth Hormone Secretion and Concentration. GH secretion and concentration levels for samples taken every 10 minutes for the entrained (A and B) and misaligned (C and D) groups. Scheduled sleep episodes are represented by the black bars. Baseline for the entrained group is represented by the black circles and samples obtained from the second sampling window after exposure to entrained sleep and wakefulness schedules are represented by the blue circles. Baseline for the misaligned group is represented by the black triangles and samples obtained from the second sampling window after exposure to misaligned sleep and wake schedules are represented by the red triangles.
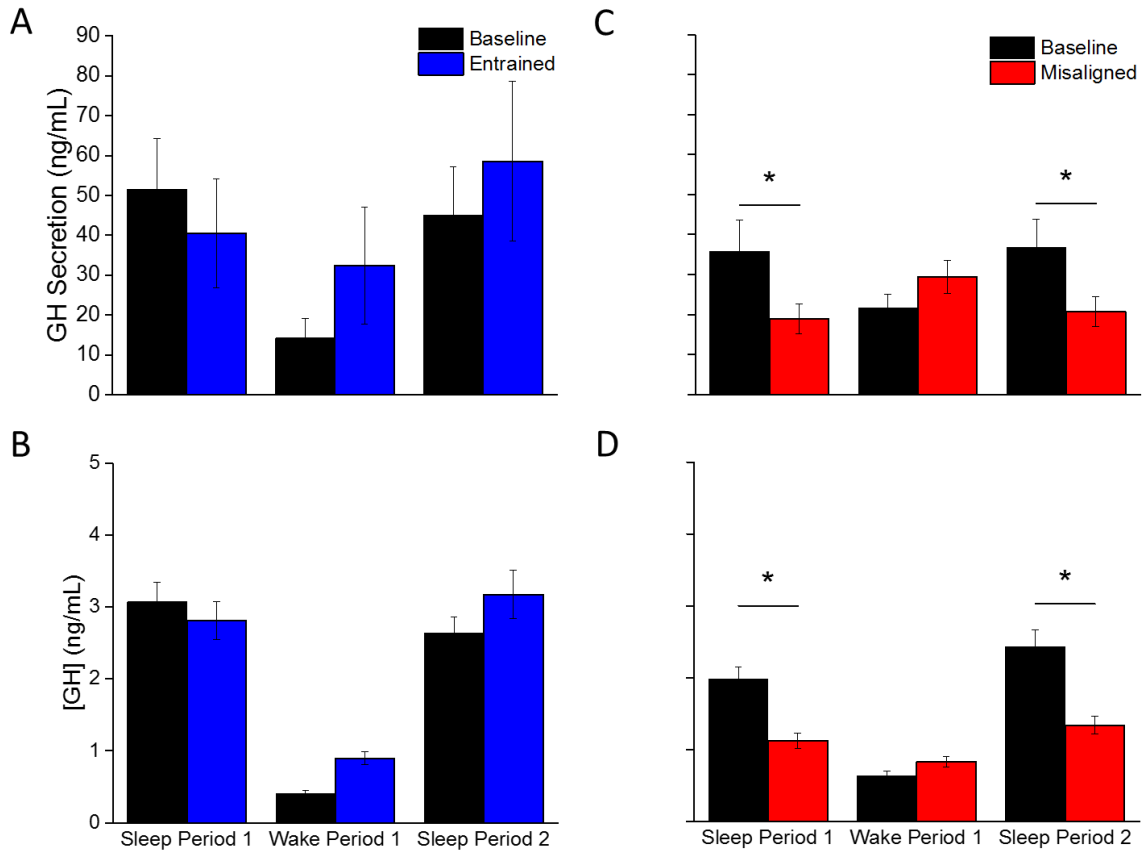
Figure 4. Growth Hormone Secretion and Concentration during Scheduled Sleep and Wakefulness. Total GH secretion and concentration during each sleep or wakefulness period for the entrained (A and B) and misaligned (C and D) groups. Scheduled sleep is represented by the horizontal black bars. Vertical black bars represent values for the baseline sampling window for each group. Blue bars represent sampling that occurred during the second sampling window under entrained conditions. Red bars represent sampling that occurred during the second sampling window under misaligned condition. Asterisks (*) represent significant differences from baseline within group ($p < 0.05$). Error bars represent ± SEM.
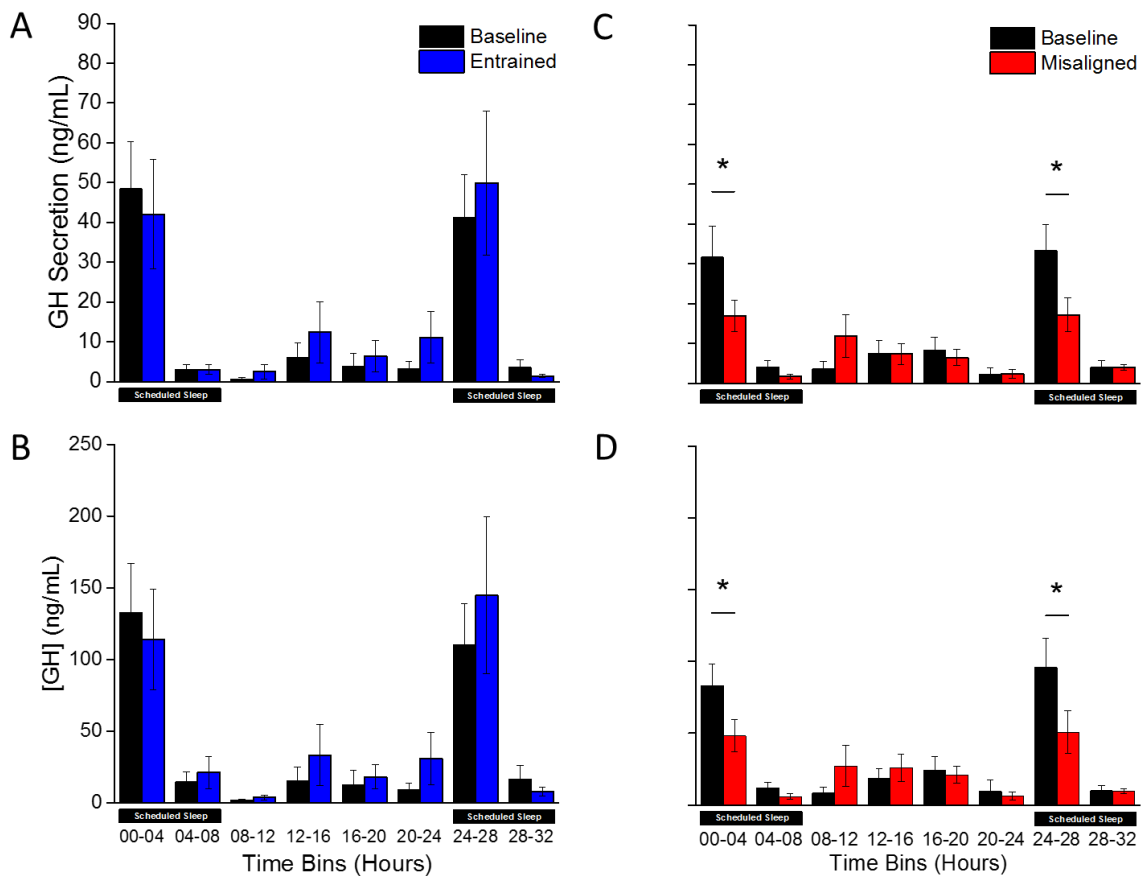
Figure 5. 4h Bins of Growth Hormone Sampling. Total GH secretion and concentration divided into 4h bins across the 32h sampling period of sleep and wakefulness for the entrained (A and B) and misaligned (C and D) groups. Scheduled sleep is represented by the horizontal black bars. Vertical black bars represent values for the baseline sampling window for each group. Blue bars represent sampling that occurred during the second sampling window under entrained conditions. Red bars represent sampling that occurred during the second sampling window under misaligned conditions. Asterisks (*) represent significant statistical differences from baseline sampling from that group ($p < 0.05$). Error bars represent $\pm$ SEM.
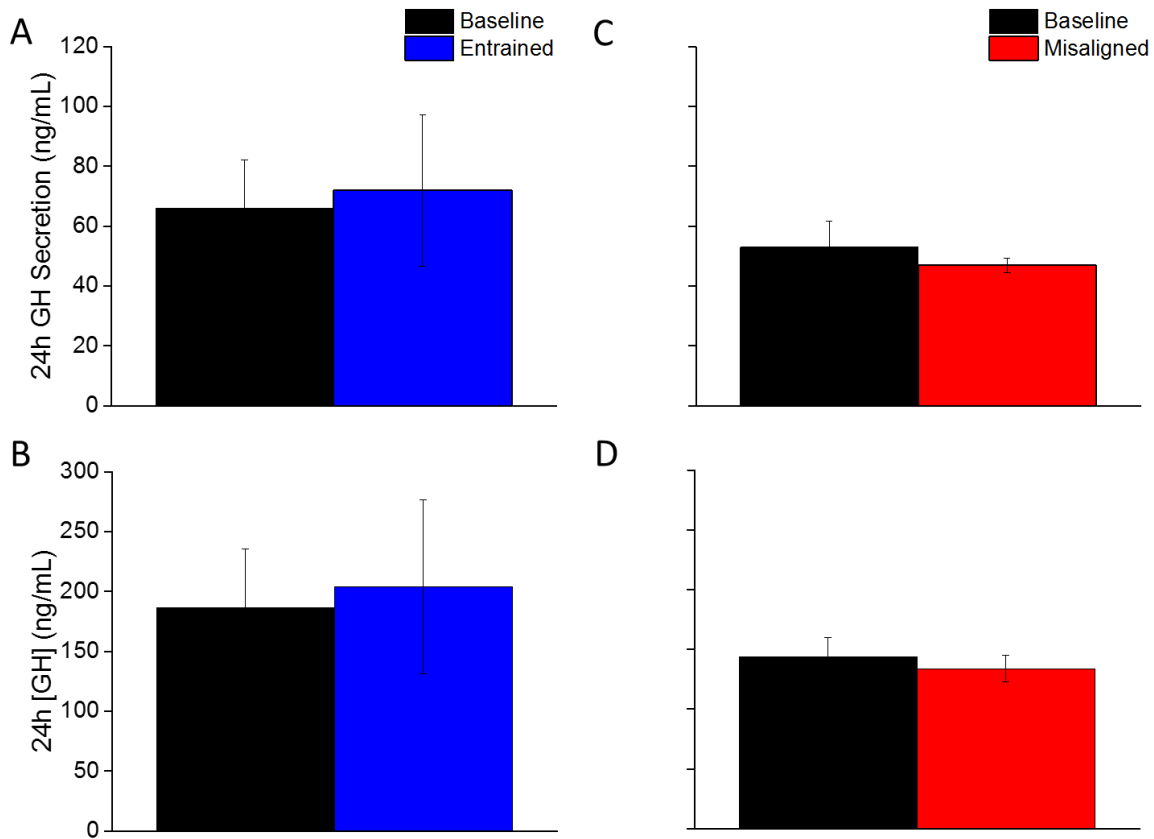
Figure 6. Total 24h Growth Hormone Secretion and Concentration. Total GH secretion and concentration for the first 24h of each sampling period for the entrained (A and B) and misaligned (C and D) groups. Black bars represent values for the baseline sampling period for each group. Blue bars represent sampling that occurred during the second sampling period under entrained conditions. Red bars represent sampling that occurred during the second sampling period under misaligned conditions. Error bars represent ± SEM.

DISCUSSION

The current findings demonstrate that chronic circadian misalignment between circadian and sleep timing impacts the temporal pattern of endocrine physiology. Specifically, growth hormone secretion was decreased during scheduled sleep episodes after exposure to weeks of circadian misalignment compared to when sleep occurred at an appropriate entrained relationship to circadian timing. This change in GH secretion levels was observed during the first half of scheduled sleep episodes for subjects in the misaligned group whereas subjects in the entrained group showed no significant change across the study duration living in the laboratory when sleep and wakefulness coincided with appropriate biological timing (i.e. sleep during the biological night, wakefulness during the biological day).

GH secretion was found to be lower in scheduled sleep episodes during chronic circadian misalignment compared to baseline entrainment, but no change was found in scheduled wakefulness. GH secretion has been shown to be primarily regulated by sleep. Specifically, GH is released in a pulsatile fashion with the largest pulse coinciding with slow wave sleep, especially in the early part of the night (2, 22, 43, 44, 48). Furthermore, pharmacological enhancement of slow wave sleep has been demonstrated to increase mean secretory rates of GH (16). In the current study, the decrease in growth hormone secretion during sleep was found to primarily be a result of decreases in secretion during the first 4-h of sleep during the typical peak secretion time, and not at any other 4-h time bin of sleep or wakefulness across the 32-h of sampling.

As no change in the amount of slow wave sleep occurred during weeks of circadian misalignment compared to baseline, circadian misalignment does not appear to primarily alter the regulation of slow wave sleep and thus there may be a mechanism controlling GH secretion

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independent of slow wave sleep. The observed decrease in growth hormone secretion without a concurrent decrease in slow wave sleep (or vice versa) is contrary to much of the evidence of their tightly controlled regulation. Findings from studies using growth hormone infusion however, have shown mixed impacts on sleep architecture (27, 30) and a weak circadian influence on growth hormone (23) has been observed. Our findings suggest that the circadian system may modulate GH secretion, coordinating sleep timing for healthy function, or in the case of dysregulation, redistributing secretion throughout the 24-h day to maintain total secretion levels. Our findings suggest that proper alignment of sleep and circadian timing is important to promote elevated GH secretion during sleep.

In this protocol, total 24h growth hormone secretion was found to be similar at baseline and reassessment regardless of circadian entrainment or misalignment. Findings from other studies have demonstrated approximately conserved total growth hormone secretion levels over 24h with total sleep deprivation (3) and sleep restriction (46). Acute shifts of sleep to daytime hours and habitual night shiftwork also shows conserved total 24-h secretion levels (56). Additionally, night shift workers with disrupted melatonin rhythms, or incomplete adaptation to their habitual night work and daytime sleep schedules also showed similar overall growth hormone levels despite significantly decreased growth hormone secretion during sleep episodes and similar amounts of slow wave sleep (4). This suggests that there may be an interaction between circadian and sleep homeostatic mechanisms which regulates growth hormone concentrations in the occurrence of sleep or circadian challenges.

It is also possible that there is an additional regulatory mechanism that mediates the relationship between sleep and growth hormone secretion, allowing for redistribution of growth hormone pulses at other times of the day to conserve total 24-h secretion levels to protect

physiological functions of growth and metabolism for which growth hormone is required. When sleep opportunities were restricted to 4-h (2-h later bedtime and 2-h earlier waketime) for 7 days, growth hormone secretion developed a biphasic pattern, with significantly secretion in the 3-h before bedtime during extended wakefulness than when 12h sleep opportunity was provided (46). Interestingly, no difference in the amount of growth hormone secreted in the first 3-h of sleep or change in slow wave sleep was observed when sleep restriction was compared with extended sleep opportunity.

The physiological implications of altered temporal pattern of GH secretion, especially lower levels during the first half of sleep are unknown, but it may contribute to other observed alterations in metabolic and immune physiology. For example, alterations in growth hormone secretion could potentially impact anabolic and metabolic processes as well as innate immunity and bone and muscle growth and maintenance. GH stimulates insulin-like growth factor 1 (IGF-1) production in the liver and regulates IGF-1 levels in the blood and tissues (15, 53). IGF-1 is an important hormone for bone growth and tissue repair (14, 55) and it can also regulate GH secretion through negative feedback (5). Therefore, the reduction in GH secretion during sleep found during circadian misalignment could have potential consequences for normal bone development and repair of tissues. Future research should examine the impact of circadian misalignment on IGF-1 and other related endocrine measures.

In the current study, we examined the impact of chronic circadian misalignment in healthy adults in a controlled laboratory setting. This exposure to chronic circadian misalignment is a failure to entrain to the scheduled sleep and wakefulness day-length. This occurs in Non-24-Hour Sleep-Wake Disorder and also has potential implications for the future of exploration class space missions such as adaptation to living on planets including as Mars. Future studies should

examine other subject populations (e.g., older adults and patients with this and other circadian rhythm sleep-wake disorders) as well as other types of circadian misalignment (e.g., early morning shift work) and chronic misalignment in night workers where they typically transition between sleeping during the day when working at night and sleeping at night on days off. Additionally, further investigation of the physiological impact of the temporal redistribution observed as conservation of overall 24h growth hormone secretion levels on metabolism and growth functions, including glucose, protein and lipid regulation, is needed.

Growth hormone secretion, in addition to large pulses at night, has additional smaller pulses that occur consistently a few hours after meals (19, 20, 35, 48). Growth hormone has been shown to regulate substrate utilization during times of fasting, decreasing glucose uptake (25, 37, 67) and upregulate energy use from lipids (38). During sleep, i.e. overnight fasting, growth hormone has been shown to increase lipolysis and lipid oxidation with increased circulating levels of free fatty acids (FFA) and ketone bodies following a single exogenous pulse of growth hormone administration (32). Additionally, there is evidence that growth hormone administration may decrease insulin sensitivity (36). This converging evidence demonstrates that growth hormone is important for regulation of substrate utilization and thus dysregulation may have consequences for energy metabolism. In the context of our findings, the decreased levels of growth hormone overnight may precipitate higher glucose levels, which could lead to decreases in insulin sensitivity. Additionally, conservation of 24h growth hormone secretion involving redistribution of growth hormone secretion to other times of sleep and wakefulness could impact glucose uptake and insulin sensitivity at other times, resulting in dysregulation of energy metabolism throughout the day and night. These findings contribute to the growing body of literature of the relationship between altered sleep timing and growth hormone secretion,

demonstrating the potential involvement of circadian mechanisms with potential implications of

metabolic and endocrine functions.

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CHAPTER 5

CONCLUSIONS

Ellen R. Stothard

Summary of Findings

The goal of this dissertation was to investigate the influence of sleep and circadian rhythms on metabolic and endocrine regulation. Current, insufficiencies exist in how circadian misalignment promotes states of weight gain obesity. First, with 20% of the US population participating in shift work, and the largest portion starting work in the early morning (Finkelstein et al, 2012), it is unknown how this altered sleep and wakefulness pattern could affect metabolic physiology. We expected that early morning shift work schedules that requires waking early during the biological night would impair metabolic function as assessed by the glucose and insulin response to a test meal, as well as diet-induced thermogenesis and energy expenditure. Therefore, we tested a model of early morning shift work to examine the impact of morning circadian misalignment on metabolic outcomes in response to a test meal. We found that a 6.5h sleep opportunity timed earlier by 1 hour (similar to unpublished data by our laboratory from early morning shift workers) significantly decreased sleep duration and resulted in awakening while melatonin levels were elevated compared to waking at habitual time. Additionally, in response to an identical meal, glucose levels were increased by ~5% in simulated early morning shift work compared to habitual wake time. No difference was observed in insulin or diet-induced thermogenesis. Finally, energy expenditure was initially lower in simulated early morning shiftwork compared to habitual wake timing prior to the meal but was not different following the meal between conditions.

Secondly, the influence of circadian misalignment on appetite and hunger is largely unknown. Obesity prevalence is increasing rapidly and recently connections to circadian and sleep disruption have come to the forefront of scientific interest due to their interactions with metabolic processes. Food intake at an adverse circadian time can lead to weight gain (Arble et

al, 2009; Baron et al, 2011) yet little research has been done to examine the role of the circadian system in hunger and appetite. Using self-reported feelings of hunger and appetite in two protocols: 1) overnight wakefulness with food intake in simulated night shift work and 2) overnight wakefulness without food intake in 40-h of total sleep deprivation, we found that overall hunger followed meal patterns such that it rose prior to food consumption and subsequently decreased. Additionally, we found that 4 factors explained ~86% of the total cumulative variance, representing the constructs of main course appetite, hunger, snacks/dessert appetite, and caffeine//hirst. During circadian misalignment in simulated night shiftwork, main course appetite, snacks/dessert appetite, hunger, and caffeine/thirst were lower during the night shifts than during the day. During overnight fasting of sleep deprivation, both types of appetite were lower whereas hunger steadily increased in the absence of food intake occurred and caffeine/thirst ratings were low.

Finally, it is largely unknown how chronic circadian misalignment influences growth hormone secretion. As growth hormone pulsatility has been closely linked to sleep architecture, the impact of circadian misalignment, specifically exposure to weeks of chronic circadian misalignment, on growth hormone was tested. We hypothesized that chronic circadian misalignment would decrease growth hormone secretion during sleep. We found that growth hormone secretion was decreased in the first 4-h of sleep after exposure to weeks of circadian misalignment compared to entrained conditions. However, this decrease was observed without changes in slow wave sleep or changes in 24-h total growth hormone secretion.

In summary, this dissertation focused on contributing to the knowledge of the influence of circadian misalignment on metabolic physiology and ratings of appetite and hunger to determine if changes may be factors that contribute to negative metabolic health outcomes. We

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showed that circadian misalignment induced by waking early in the morning under conditions of simulated early morning shift work increases glucose without a compensatory response of insulin, which could increase risk of diabetes and obesity in workers with chronic exposure. Furthermore, overnight wakefulness with and without food intake show decreases in hunger and appetite. This suggests that if food intake occurs in overnight shift workers, it is not due to increases in appetite or hunger. Finally, weeks of exposure to circadian misalignment decreases growth hormone secretion in the first 4-h of sleep compared to weeks of entrainment without a decrease in slow wave sleep or 24-h total secretion.

Future Directions

These findings improve the understanding of metabolic and endocrine physiology and how circadian misalignment maybe contribute to metabolic dysregulation. Future studies could build on our findings in the following ways: First, our simulated early morning shift work model tested young, healthy individuals [Age 23.0 ± 3.5y ($\pm$SD) BMI 23.5 ± 2.0] using a test meal. As shift workers are more likely to be overweight or obese and have increased rates of cormorbid disease (Karlsson et al, 2001; Bushnell et al, 2010), studying other populations including habitual shift workers, overweight or obese individuals and other clinical populations is needed. Additionally, use of intravenous glucose tolerance testing (IVGTT), oral glucose tolerance testing (OGTT), or hyperinsulinemic/euglycemic clamp procedures could provide additional information into the mechanisms underlying our finding of increased glucose in response to a test meal.

Additionally, though our study found circadian misalignment to reduce appetite and hunger, even in the presence and absence of food intake overnight, this was shown using self-

reported hunger ratings. Further research is needed employing other methods of assessing hunger such as buffet-style food selection or *ad libitum* food availability. Additionally, tasks using pictures or food smells or tastes could be employed to learn more about the involvement of the sensory aspect of hunger and food intake.

Finally, our study of growth hormone secretion occurred under controlled laboratory research conditions in an environment free of time cues. Future research could combine more real-world conditions such as variable lighting or meal timing to see if these inputs mediate the circadian misalignment effects on growth hormone.

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