CONSEQUENCES OF CHRONIC SLEEP RESTRICTION ON ENERGY BALANCE IN HEALTHY ADULTS

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A DISSERTATION

in

Psychology

Presented to the Faculties of the University of Pennsylvania

in

Partial Fulfillment of the Requirements for the

Degree of Doctor of Philosophy

2014

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IN HEALTHY ADULTS

ACKNOWLEDGMENT

I would like to thank my advisors, David Dinges and Namni Goel for all of their support during the past three years. They have always been exceedingly generous with their time as they guided me through my training as a graduate student. I feel lucky to have mentors who were able to help me embrace the big-picture aspects of research and a career in academia as well as the importance of the fine details involved in writing and giving presentations effectively. They have both also led by example; I greatly respect their work ethic and have enjoyed working in such a productive environment that holds research to the highest scientific and ethical standards. The entire faculty and staff in the Unit for Experimental Psychiatry have been so helpful; particularly Michele Carlin, the Project Research Manager, who helped me add energy balance measurements to our existing protocols and taught me how to successfully run in-laboratory studies with human subjects.

I would also like to thank my family for their continued support of my career goals. I am so lucky to have parents who instilled in me the value of education and ambitious drive and a sister who is always there to talk to and make me laugh when I need her. Since meeting in college during my senior year at Elon, my fiancé Brian has shown infinite encouragement as my studies have taken me from North Carolina to Virginia and then Pennsylvania. Brian has helped me work through experiment ideas, listened to rehearsals of presentations, and read drafts of manuscripts. Most importantly, he (with the help of Lucy and Molly) is always able to make me happy, even after a long stressful and frustrating day at the lab. I know with certainty that I would not be where I am today without Brian and my family.

Finally, I would like to thank the Grill lab — my training related to the neural and physiological basis for energy balance was immeasurably useful during my dissertation research, as well as my committee members Lori Flanagan-Cato and Irwin Lucki for their support and guidance during my doctoral training. I feel fortunate to have had the opportunity to attend lectures and seminars at this prestigious institution and learn from such renowned professors.

ABSTRACT

CONSEQUENCES OF CHRONIC SLEEP RESTRICTION ON ENERGY BALANCE IN HEALTHY ADULTS

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Habitual short sleep duration is consistently associated with weight gain and increased risk for obesity. The objective of this dissertation was to elucidate how chronic sleep restriction impacts components of energy balance, namely, weight gain, energy intake, and energy expenditure. Healthy adults (21-50 y) participated in controlled isolated laboratory protocols for 14-18 days and were randomized to either an experimental condition (baseline sleep followed by sleep restriction [5 consecutive nights of 4 hours time-in-bed [TIB] per night] and recovery sleep) or control condition (no sleep restriction: 10 hours TIB per night for all nights). Sleep-restricted subjects exhibited significant weight gain, increased caloric intake, greater consumption of fat, delayed meal timing and decreased resting metabolic rate (the largest component of energy expenditure) during sleep restriction but these changes returned to baseline levels after one night of recovery sleep (12 hours TIB). Control subjects did not exhibit a significant change in weight, caloric intake or resting metabolic rate across corresponding protocol days. Notably, there were significant gender and race differences in the energy balance response to sleep restriction. Men gained more weight, increased caloric intake to a greater degree during sleep restriction and consumed a larger percentage of calories during late-night hours than women. Relative to Caucasians, African Americans consumed a comparable amount of calories during baseline and sleep restriction but exhibited marked energy expenditure deficits after baseline sleep, sleep restriction and recovery sleep, and gained more weight during the study. In the largest, most diverse healthy sample of adults studied to date under controlled laboratory conditions, sleep restriction promoted weight gain and positive energy balance. Collectively, these results highlight the importance of obtaining sufficient sleep for regulating energy balance and maintaining a healthy weight, particularly in men and African Americans.

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CHAPTER 1: INTRODUCTION

'Sleep persists in predators and prey, carnivores and vegetarians, on the land and in the water, in most mammals as they lie down relaxed, in ruminants while they stand, in birds while they perch, and in dolphins which constantly swim … in the smartest and in the dumbest of all mammalian species' (Rechtschaffen, 1998). Sleeping, like eating, is a biological imperative that consumes approximately one-third of human life. Just as missing a day's worth of meals produces strong feelings of hunger, being deprived of a night's sleep leads to overwhelming feelings of fatigue. Sleep resists being deprived; if exhausted enough, humans can fall asleep under even dangerous circumstances (Nansen, 1899). When sleep is denied, there is an increase in the frequency and duration of sleep episodes, and an elevation in the intensity of sleep (Banks et al., 2010; Borbely and Tobler, 1996; Brunner et al., 1993; Goel et al., 2009a; Van Dongen et al., 2003).

These properties of sleep and its ubiquitous manifestation across species (Allada and Siegel, 2008; Siegel, 2008) indicate that sleep serves an evolutionarily adaptive purpose. Siegel has argued that sleep increases the efficiency of behavior by regulating its timing – sleep occurs when there is maximal predator and injury risk and minimal opportunity for meeting vital needs (such as eating and mating) – and by conserving energy during periods of inactivity (brain and body metabolism are decreased during sleep) (Siegel, 2009). Sleep is also needed to replenish energy stores (e.g. glycogen) in the brain that are depleted during wakefulness (Scharf et al., 2008; Porkka-Heiskanen and Kalinchuk, 2011) and to restore synaptic and cellular homeostasis after synaptic plasticity occurs during learning and interacting with the environment (Tononi and Cirelli, 2014). Most humans who experience sleep loss; either acute total sleep deprivation (e.g., 1-2 consecutive nights of no sleep) or chronic partial sleep deprivation (e.g. ≥ 3 consecutive nights of limited sleep, typically ≤ 6 hours), exhibit profound neurobiological and physiological changes that can impede performance and negatively impact health. This dissertation focuses on how sleep restriction impacts energy balance in order to better understand why short sleep duration is associated with obesity in humans.

HEALTHY HUMAN SLEEP

Normal human sleep is comprised of two states—rapid eye movement (REM) and non-REM (NREM) – that alternate cyclically during a sleep episode. Using electroencephalography (EEG), characteristics of these sleep states have been well defined (Rechtschaffen and Kales, 1968; Silber et al., 2007). NREM sleep shows synchronous cortical EEG (sleep spindles, K-complexes, and slow waves) and is associated with low muscle tone and minimal psychological activity whereas REM sleep shows desynchronized EEG and is associated with muscle atonia and dreaming (Kryger et al., 2011). In healthy humans, the timing, intensity and duration of sleep is primarily regulated by two processes: homeostatic regulation and circadian timing (Borbely, 1982; Borbely, 1998).

Sleep homeostasis describes the drive for sleep that increases progressively during wakefulness and decreases progressively during NREM sleep (Borbely, 1994). The propensity for sleep is increased after prolonged wakefulness and decreased after prolonged sleep. Similarly, sleep restriction leads to an increase in the intensity and duration of subsequent sleep, whereas prolonged sleep leads to decreased sleep intensity and duration (Borbely, 1982). Slow wave, low-frequency electroencephalography (EEG); likely represent a measure of sleep intensity – extended waking leads to increases in slow-wave energy (SWE) during the subsequent recovery night and the extent of this SWE increase is a function of prior wake duration (Åkerstedt et al., 2009; Brunner et al., 1990). In addition to SWE, other biological markers of sleep homeostasis have been identified including extracellular adenosine, central nitrous oxide levels and salivary amylase; levels of these markers increase with prolonged sleep wakefulness and thus may reflect an increased sleep drive (Kalinchuk et al., 2006; Kalinchuk et al., 2011; Scharf et al., 2008; Seugnet et al., 2006). The homeostatic process of sleep-wake regulation interacts with but is independent from circadian control (Dijk et al., 1989b).

The circadian process that controls sleep is described as the 24-hour oscillatory variation in the propensity for sleep. This 24-hour period is the time it takes the Earth to rotate about its own axis which generates daily environmental cycles of ambient temperature and illumination.

The alternation of light and darkness directly entrains an organism's circadian rhythms and thus influences its life patterns, creating species that are active primarily during the light (diurnal), the dark (nocturnal), twilight periods (crepuscular) or during the light and the dark (cathemeral) (Pittendrigh, 1981). Environmental light is transmitted from the retina to suprachiasmatic nuclei in the anterior hypothalamus which then transmit this information to the pineal gland via a multisynaptic pathway; the pineal gland secretes melatonin, a hormone that regulates various biological functions (Czeisler, 1995; Lucas, et al., 1999; Ralph et al., 1990; Sadun et al., 1984; Watts, 1991). In humans, melatonin increases during the dark cycle which coincides with a period of inactivity and sleepiness and decreases during the light cycle which coincides with a period of activity and wakefulness (Shanahan and Czeisler, 1991). This circadian process interacts with but is independent from sleep homeostasis (Åkerstedt and Froberg, 1978).

Scientific evidence shows that human sleep is naturally regulated by these two processes, with sunrise and sunset providing the photic signals necessary to entrain the sleep-wake cycle (Wehr et al., 2001). However, with the introduction of artificial light and other technologies, television and alarm clocks have replaced these natural signals and thereby may not allow optimal sleep duration. Additionally, industries that operate 24 hours a day produce light and noise that can interfere with sleep (Basner et al., 2011) and require employees to work during hours usually devoted to sleep (i.e. shift work) (Schoenborn and Adams, 2010). Finally, with the increase in international business and air travel, many adults frequently travel across time zones, affecting the circadian timing system. Thus, many humans are challenging basic biological pressures in order to accommodate social norms and obligations (Wittmann et al., 2006).

For most healthy adults, physiological sleep duration ranges between 7.0 and 8.5 hours (Krueger and Friedman, 2009); however, habitual sleep duration among adults is determined by a variety of factors and shows considerable variance within and between individuals (Van Dongen et al., 2005). Data from the 2005-2008 National Health and Nutrition Examination Survey suggest that self-reported sleep duration is distributed approximately normally and 2004-2007 National Health Interview Survey data reveal that 7.8% of adults report sleeping less than 5 hours per

night, 20.5% report sleeping 6 hours per night, 30.8% report sleeping 7 hours per night, 32.5% report sleeping 8 hours per night and 8.5% of adults report sleeping more than 9 hours per night (Krueger and Friedman, 2009). Among employed American adults, two epidemiological studies found that the prevalence of being a short sleeper (either ≤6 hrs/day or <6 hrs/day) has increased significantly in the past few decades (Knutson et al., 2010; Luckhaupt et al., 2010). Studies have suggested that habitual short sleepers do not require less sleep than adults who typically sleep 7-8 hours per night; rather, these short sleepers gradually accrue a sleep debt over time (Aeschbach et al., 1996; Bradshaw et al., 2007; Klerman and Dijk, 2005). Evidence from the American Time Use Survey indicates that adult sleep duration is significantly shorter during weekdays compared to weekends (Basner et al., 2007) suggesting that adults attempt to recover sleep debt accrued during the week by extending sleep when it is presumably more convenient and when schedules are likely more flexible.

CAUSES OF INSUFFICIENT SLEEP

Compensated work time is the most potent determinant of sleep duration (Basner et al., 2007). There is a higher prevalence of short sleep duration among full-time employed adults when compared to part-time workers, students, retired individuals, homemakers or unemployed adults (Knutson et al., 2010; Luckhaupt et al., 2010) and longer work hours are associated with shorter sleep duration (Hale, 2005; Nakashima et al., 2010; Virtanen et al., 2009). Adults working 8 hours or more per day have the same bedtime compared to adults who work less than 8 hours per day but wake up 0.68 hours earlier (Basner and Dinges, 2009). Shift work schedules often require work to occur during the night and require sleep to occur during the day which creates a conflict between the worker's internal circadian rhythm and drives for sleep/wake and his/her required sleep-wake schedule (Åkerstedt, 2005; Kolla and Auger, 2011). Some individuals (e.g. college students, medical residents, military personnel, truck drivers and shift workers) undergo prolonged periods of wakefulness without sleep ('pulling all-nighters'; acute total sleep

deprivation) in order to meet deadlines for school or as part of work duties (Baldwin and Daugherty, 2004; Philip et al., 2002; Thacher, 2008; Wright et al., 2013).

Travel time (composed of traveling to work [commute], stores, schools and social events) is negatively associated with sleep time (Basner et al., 2007). According to the Census American Community Survey Report (2011) and the American Times Use Survey (Christian, 2012), American workers spend nearly an hour commuting to and from work each day and 33.5% of workers have commutes that are greater than 30 minutes each way. A majority of Americans (55.4%) leave their homes between 0600h – 0830h in order to arrive at work on time (Census American Community Survey Report, 2011) and they do not arrive home until late in the evening in part due to traffic. The result is an increase in the length of the work day and a decrease in time for other activities including sleep.

Although humans are diurnal, some individuals prefer activity in the morning (larks) whereas others prefer activity in the evening (owls). Morning-type and evening-type individuals differ endogenously in the circadian phase of their biological clocks (Baehr et al., 2000; Kerkhof and Van Dongen, 1996). Work schedules of industrial societies usually complement individuals who function best in the morning; owls often accumulate sleep debt during the work week when they are alert late at night but must wake early for work (Korczak et al., 2008; Roenneberg et al., 2003; Roepke and Duffy, 2010; Taillard et al., 2003).

CONSEQUENCES OF INSUFFICIENT SLEEP

Neurobehavioral Performance

According to the National Transportation Safety Board (1989; 1995), 30-40% of all US truck accidents are fatigue related and adults obtaining insufficient sleep are more likely to be drowsy drivers (Maia et al., 2013) and are at increased risk for crashing (Abe et al., 2010; Martiniuk et al., 2013). Several catastrophes including the Exxon Valdez accident, Chernobyl and Three Mile Island nuclear plant meltdowns, and Space Shuttle Challenger tragedy were partially due to human error resulting from sleepiness and fatigue (Mitler et al., 1988). Sleepiness due to

long work hours has also been implicated in workplace errors among medical professionals (Barger et al., 2006; Czeisler, 2009; Lockley et al., 2004), police officers (Rajaratnam et al., 2011; Vila, 2006) and individuals in the military (Giam, 1997; Hartzler, 2014; Lieberman et al., 2005; Lieberman et al., 2006).

Distinct sleep/wake-related physiological processes interact to regulate alertness and neurobehavioral performance (Raslear et al., 2011; Van Dongen and Dinges, 2005). Sleep homeostatic process S, which tracks recent sleep history (Daan et al., 1984) and process U, which monitors sleep-wake on a longer time scale (nightly sleep duration) (McCauley et al., 2013; McCauley et al., 2009) affect performance by increasing the propensity for sleep as hours of wakefulness increase across the day or over several days of prolonged wakefulness. The endogenous circadian process (process C) tracks changes in light exposure (as well as other zeitgebers or synchronizers) and entrains sleep propensity to the light-dark cycle: when sleep propensity is increased (during the night) waking performance is degraded (Czeisler et al., 1999).

In laboratory studies, sleep restriction (consecutive nights of sleep limited to less than 6 hours per night) leads to increased sleep propensity, decreased alertness and deficits in sustained attention, cognitive throughput and working memory in healthy adults (Axelsson et al., 2008; Banks et al., 2010; Belenky et al., 2003; Bliese et al., 2006; Carskadon and Dement, 1981; Cote et al., 2008; Dinges et al., 1997; Fafrowicz et al., 2010; Goel et al., 2009a; Guilleminault et al., 2003; Pejovic et al., 2013; Rupp et al., 2009; Van Dongen et al., 2003; Wu et al., 2010). As the need for sleep increases across nights, performance becomes progressively more impaired and unstable (Doran et al., 2001). Many of the deficits caused by sleep restriction persist even after a night of 10 hours recovery sleep (Banks et al., 2010) or several nights of 8 or 10 hours recovery sleep (Axelsson et al., 2008; Belenky et al., 2003; Pejovic et al., 2013; Rupp et al., 2009). However, “banking sleep” by spending 10 hours in bed each night for one week prior to experiencing sleep restriction attenuates neurobehavioral performance deficits during sleep restriction and facilitates improvement after recovery sleep (Rupp et al., 2009).

Physiology and Health

Self-reported habitual short sleep duration is associated with poor health (Geiger et al., 2012; Steptoe et al., 2006) and may be a risk factor for mortality (Cappuccio et al., 2010b; Gallicchio and Kalesan, 2009; Kurina et al., 2013) in adults. Cross-sectional and prospective population studies indicate that short sleep duration is also associated with weight gain (Appelhans et al., 2013; Chaput et al., 2008; Lyytikainen et al., 2011; Mozaffarian et al., 2011; Xiao et al., 2013), higher BMI (Ford et al., 2014; Kobayashi et al., 2012) and obesity (Bo et al., 2011; Cappuccio et al., 2008; Di Milia et al., 2013; Theorell-Haglow et al., 2012; Yiengprugsawan et al., 2012). The relationship between short sleep duration and weight gain/obesity has also been observed among children and adolescents with odds ratios typically larger than those calculated in adults (Al-Hazzaa et al., 2012; Chen et al., 2014; Ekstedt et al., 2013; Magee and Lee, 2014; Magee et al., 2013; Mitchell et al., 2013; Moraleda-Cibrian and O'Brien, 2013; Pileggi et al., 2013; Suglia et al., 2013).

Short sleep duration has also been identified as a risk factor for diseases associated with obesity such as cardiovascular disease (Buxton and Marcelli, 2010; Cappuccio et al., 2011; Eguchi et al., 2012; Gangwisch et al., 2013; Iglayreger et al., 2014; Knutson, 2010; Magee et al., 2012; Shankar et al., 2008), hypertension (Chaput et al., 2013; Faraut et al., 2012; Gangwisch et al., 2013; Guo et al., 2013; Stranges et al., 2010; Zou et al., 2013) and type 2 diabetes (Beihl et al., 2009; Cappuccio et al., 2010a; Kita et al., 2012; Knutson and Van Cauter, 2008; Lou et al., 2012; McNeil et al., 2013; Merikanto et al., 2013; Najafian et al., 2013).

Experimental studies demonstrate that sleep loss causes various physiological changes that may underlie the relationship between short sleep duration and poor health/increased risk for the aforementioned diseases. These effects include: changes in appetite regulating hormones (e.g., ghrelin) (Nedeltcheva et al., 2010; Schmid et al., 2008; Spiegel et al., 2004b), decreased glucose tolerance and insulin sensitivity (Buxton et al., 2010; Nedeltcheva et al., 2009a; Spiegel et al., 1999), elevated blood pressure (Carter et al., 2012; Lusardi et al., 1996; Ogawa et al., 2003; Robillard et al., 2011), increased sympathetic activity and venous endothelial dysfunction

(Dettoni et al., 2012), augmented pro-inflammatory responses (Meier-Ewert et al., 2004; van Leeuwen et al., 2009; Vgontzas et al., 2004) and an attenuated immune response to vaccination (Lange et al., 2003; Prather et al., 2012; Spiegel et al., 2002).

Sleep and Energy Balance

The finding that sleep loss is associated with weight gain in humans is counterintuitive given that one proposed function of sleep is to conserve energy – more energy is expended when awake than when asleep (Penev, 2012) (**Figure 1.1**). If diet is held constant, the increased energy requirement associated with sleep restriction will lead to negative energy balance and weight loss over time (Penev, 2012). Due to an evolutionary history involving limited food availability, humans have evolved numerous mechanisms designed to defend against negative energy balance by promoting energy intake and storage (Bellisari, 2008). It is hypothesized that the body employs neuroendocrine, metabolic and behavioral compensatory responses to increase energy intake and conserve energy and offset the increased energy requirement associated with sleep restriction (Penev, 2012).

Several laboratory studies show support for this theory. Energy expenditure is greater during both acute total (Jung et al., 2011) and partial (Markwald et al., 2013; Shechter et al., 2013) sleep deprivation compared to during sleep. In addition, sleep loss leads to changes in appetite regulating hormones (e.g., ghrelin) (Nedeltcheva et al., 2010; Schmid et al., 2008; Spiegel et al., 2004b), brain activity in areas related to reward (Benedict et al., 2012; St-Onge et al., 2012a; Greer et al., 2013; St-Onge et al., 2014), metabolism (Nedeltcheva et al., 2010; Benedict et al., 2011; Buxton et al., 2012) and physical activity (Bromley et al., 2012; Schmid et al., 2009) that promote increased energy consumption and conservation.

These evolutionary mechanisms can be maladaptive in today's obesogenic environment (Swinburn et al., 1999). The increased availability of unhealthy, calorically dense and inexpensive food/drink combined with decreased physical activity due to sedentary work environments, leisure activities and modes of transportation has created an environment that promotes weight gain in the United States (Sinburn et al., 1999; Chaput et al., 2011b; Giskes et al., 2011). The extended

wakefulness associated with sleep restriction allows individuals more time to consume food/drink in this obesogenic setting. Thus, if more energy is consumed and conserved than necessary to counteract the additional energy cost of extended wakefulness, a state of positive energy balance and weight gain will occur over time (**Figure 1.2**).

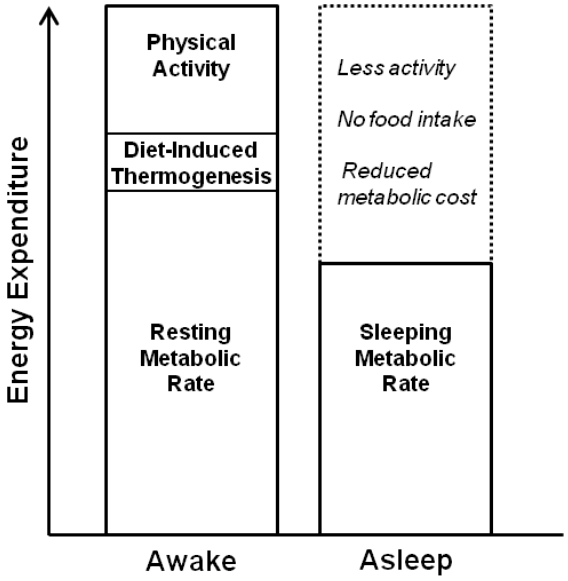

FIGURE 1.1: Additional Energy Required During Extended Wakefulness

Sleep is a state of reduced energy expenditure due to sleep-imposed immobility and lack of caloric intake, which eliminates the energy cost of physical activity and diet-induced thermogenesis. In addition, metabolic rate is reduced during sleep because less energy is needed to support brain function, sympathetic activity, breathing, circulation, and core body temperature during sleep than during wake. *Adapted from Penev, 2012.*

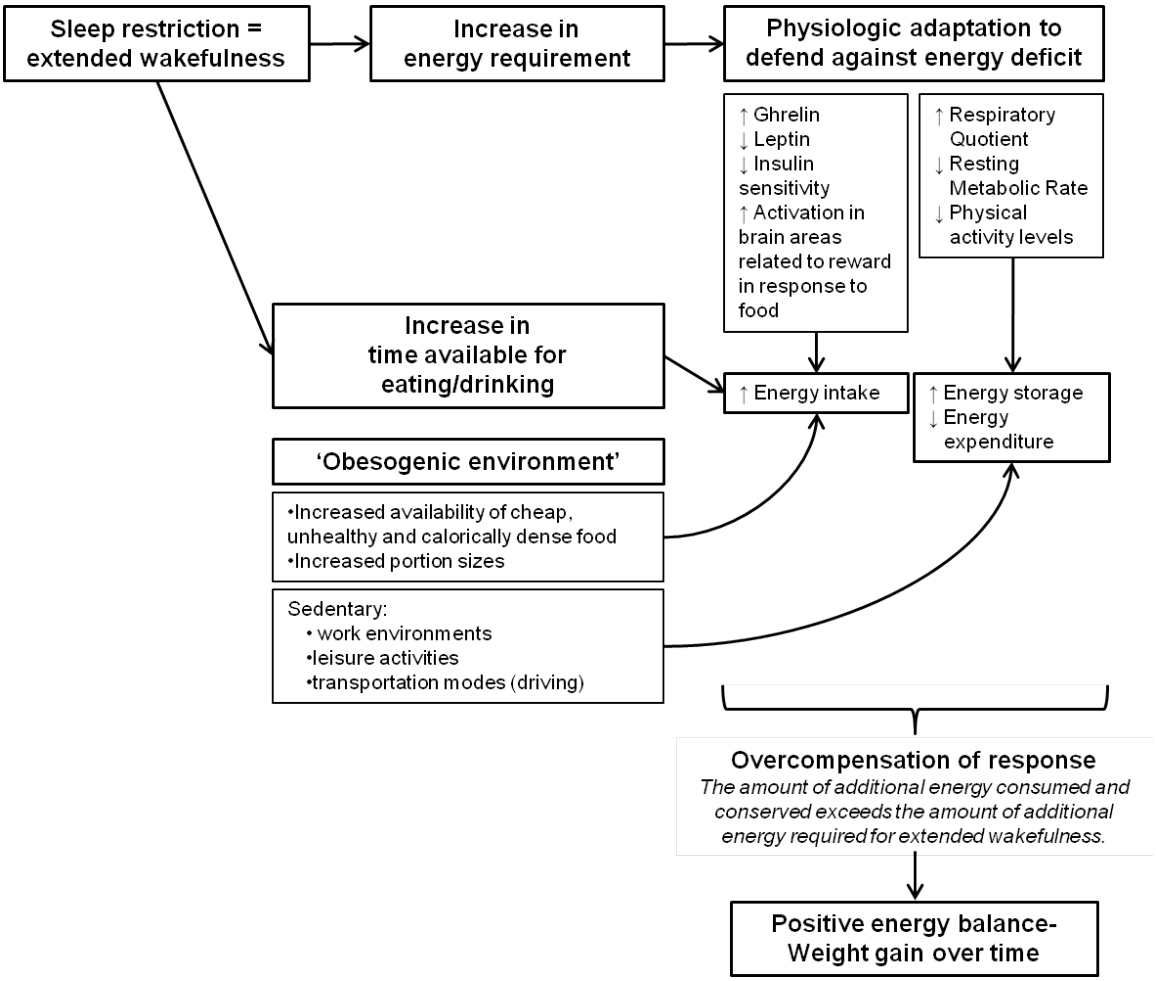

FIGURE 1.2: Proposed Model Describing How Sleep Restriction Leads to Weight Gain

This illustration provides an explanation as to why epidemiological studies consistently find that short sleep duration is associated with weight gain and obesity. It is hypothesized that the additional energy cost associated with extended wakefulness triggers a set of neuroendocrine, metabolic and behavioral responses that promote energy intake and energy storage (Penev, 2012). This response can be maladaptive within an obesogenic setting—if an individual overcompensates by consuming and conserving more energy than required for extended wakefulness, positive energy balance and weight gain will occur over time.

Gender and Race Differences in Sleep Parameters

Gender and race differences in sleep duration have been observed in population studies. When sleep is objectively measured (using actigraphy), sleep duration tends to be longer and sleep quality is higher in women than men (Jean-Louis et al., 2000; Lauderdale et al., 2006; van den Berg et al., 2009); however, women tend to self-report more sleep complaints than men (Basner, et al., 2007; Leng et al., 2014; van den Berg, et al., 2009). When sleep is measured subjectively and objectively (using actigraphy), African Americans exhibit a higher prevalence of short sleep duration, poorer sleep efficiency and report more sleep complaints (Ertel et al., 2011; Grandner et al., 2010b; Hale and Do, 2007; Jean-Louis et al., 2000; Lauderdale et al., 2006; Maslowsky and Ozer, 2013; Patel et al., 2010; Singh et al., 2005; Stamatakis et al., 2007; Whinnery et al., 2014).

Studies assessing sleep architecture using polysomnography (PSG) also show gender and race differences. Women tend to exhibit a higher percentage of slow-wave sleep and/or more delta wave activity than men (Dijk et al., 1989a; Monk et al., 1992; Armitage, 1995; Mourtazaev et al., 1995; Ehlers and Kupfer, 1997; Hoch et al., 1997; Fukuda et al., 1999; Armitage et al., 2001; Carrier et al., 2001; Redline et al., 2004; Latta et al., 2005; Bixler et al., 2009). Pubertal development is associated with a rapid decline in the duration of slow-wave sleep in adolescents (Cambell et al., 2012) and slow-wave sleep duration is reduced in menopausal women and women taking oral hormonal contraceptives (Baker et al., 001; Bixler et al., 2009). African Americans tend to exhibit a lower percentage of slow-wave sleep and less delta wave activity than Caucasians (Profant et al., 2002; Stepnowsky et al., 2003; Hong et al., 2005; Mezick et al., 2008; Hall et al., 2009; Mokhlesi et al., 2011; Song et al., 2011; Tomfohr et al., 2012). EEG spectral power analysis provides a quantification of sleep depth/intensity and delta activity (quantified by EEG spectral power in the low-frequency range) is the measure of the intensity of slow-wave sleep. Thus, women and Caucasians tend to have deeper, more intense sleep than men and African Americans, respectively. Although sex hormones have been identified as one

factor underlying the observed gender differences in sleep architecture, factors underlying the observed race differences have yet to be identified (Knutson, 2013).

Gender and Race Differences in the Consequences of Insufficient Sleep

Healthy adults show varying degrees of neurobehavioral vulnerability to sleep loss. Across nights of either total or partial sleep deprivation, a number of adults exhibit profound deficits on tests of sleep propensity and vigilant attention, others exhibit intermediate deficits on these assessments and still other 'resistant' individuals do not exhibit any change in performance (Van Dongen et al., 2004). These inter-individual differences are stable and trait-like, but have not been accounted for by demographic factors, including gender and race (Goel and Dinges, 2011). Similarly, gender and race differences have not been reported in the prevalence of car accidents or workplace errors due to sleep loss.

Conversely, gender and race differences have been found in the health consequences of insufficient sleep. Several epidemiological studies have shown a stronger association between short sleep duration and elevated BMI in men than women (Ko et al., 2007; Meyer et al., 2012; Yang et al., 2013). This pattern has also been observed in adolescents (Araujo et al., 2005) and in children (Shi et al., 2010; Tatone-Tokuda et al., 2012). Using self-report surveys, two recent epidemiological studies found that sleep duration was inversely associated with BMI in men only whereas poor sleep quality was positively associated with BMI in women only (Meyer, et al., 2012; Yang, et al., 2013). In a prospective cohort study, short sleep duration (<5 and 5-6h per night) was associated with weight gain and the development of obesity at one-year follow-up in men but not in women (Watanabe et al., 2010). Only two epidemiological studies have focused on race differences in the relationship between sleep duration and BMI; both found that the association between short sleep duration and increased odds for obesity was stronger in African Americans than Caucasians (Donat et al., 2013; Grandner et al., 2014). Gender and race differences have also been observed in the association between short sleep duration and increased risk for hypertension (Carter et al., 2012; Dean et al., 2012; Fang et al., 2012; Mezick et al., 2012; Pandey et al., 2013; Stranges et al., 2010; Wang et al., 2011) and inflammation

(Miller et al., 2009; Gamaldo et al., 2013; Grandner et al., 2013; Liu et al., 2014) with women and African Americans at higher risk than men and Caucasians respectively.

SPECIFIC AIMS OF DISSERTATION

Specific Aim 1: Determine the effects of chronic sleep restriction on weight change and assess if there are gender or race differences in this response.

Although epidemiological studies consistently find that short sleep duration associates with weight gain (Appelhans et al., 2013; Chaput et al., 2008; Lyytikainen et al., 2011; Mozaffarian et al., 2011; Xiao et al., 2013), they cannot determine the direction of the relationship in order to show that sleep restriction *causes* weight gain. For example, it is possible that the state of positive energy balance/weight gain leads to shortened sleep duration. When I began analyzing weight gain data in our subjects, no controlled laboratory studies had experimentally examined the effect of sleep restriction on weight gain in a large, diverse sample of men and women. In chapter 2 of this dissertation, I measured the change in weight (from admittance to discharge) in subjects participating in isolated laboratory protocols with ad libitum access to food/drink who were randomized to an experimental condition (including 5 consecutive nights of sleep restricted to 4 hours per night) or control condition (no sleep restriction, 10 hour sleep opportunity per night). In addition, I compared the change in weight between men and women and between Caucasians and African Americans. In chapter 4 of this dissertation, I also measured the change in weight from the day following the first baseline night of sleep to the day following the fifth night of either sleep restriction or control sleep in a separate sample of subjects.

Specific Aim 2: Determine the effects of chronic sleep restriction on measures of energy intake and energy expenditure and assess if there are gender or race differences in these responses.

After establishing that sleep restriction causes weight gain in healthy adults (dissertation chapter 2), I focused on understanding what behavioral and physiological mechanisms might underlie this effect. Specifically, I measured energy intake and two measures of energy

expenditure (resting metabolic rate and diet-induced thermogenesis). When I began recording what subjects ate/drank in our protocols, only two other laboratory studies had assessed how chronic sleep restriction affects caloric intake (Nedeltcheva et al., 2009b; St-Onge et al., 2011). Neither of these studies examined how caloric intake changes across consecutive days of sleep restriction (Nedeltcheva et al., 2009 averaged intake across all days of habitual sleep/sleep restriction; St-Onge et al., 2011 only examined caloric intake on the fifth day following habitual sleep/sleep restriction because food/drink intake was controlled during the first four days), systematically assessed how sleep restriction impacts meal timing, or determined if there were gender or race differences in the caloric intake response to sleep restriction. In chapter 2 of this dissertation I measured the daily caloric intake of subjects participating in isolated laboratory protocols with ad libitum access to food/drink. This allowed me to analyze changes in daily caloric intake, meal patterns, macronutrient content and meal timing across days following two baseline nights of sleep, five nights of sleep restriction and two nights of recovery sleep (or during corresponding days in the control condition). In chapter 3 of this dissertation, I compared caloric intake, macronutrient content and meal timing between men and women and between Caucasians and African Americans during days following baseline sleep and sleep restriction.

Although it is known that energy expenditure is greater when awake than when asleep (**Figure 1.1**), whether or not energy expenditure is altered during the day following sleep restriction remains unclear. When I began collecting measures of energy expenditure in our subjects, two studies had shown that chronic sleep restriction decreased resting metabolic rate (Nedeltcheva et al., 2010; Buxton et al., 2012) and two studies had shown that chronic sleep restriction did not change resting metabolic rate (Nedeltcheva et al., 2009b; St-Onge et al., 2011). In addition, gender and race differences in the energy expenditure response to sleep restriction had not been evaluated. In chapter 4 of this dissertation I measured resting metabolic rate, diet-induced thermogenesis and respiratory quotient during days following one baseline night of sleep, five nights of sleep restriction and one night of recovery sleep (or during corresponding days in the control condition) in subjects participating in isolated laboratory protocols with ad libitum

access to food/drink. I also compared these measurements between men and women and between African Americans and Caucasians.

Specific Aim 3: Determine the effects of short term fasting on neurobehavioral performance during sleep restriction.

There is evidence to suggest that energy balance and sleep-wake are two interacting homeostatic systems (Vanitallie, 2006). Not only does sleep duration influence energy balance (the focus of dissertation chapters 2-4), but energy balance also influences the sleep-wake system. Caloric intake, particularly fat consumption, leads to decreased arousal and increased sleepiness in animals and humans (Danguir, 1987; Hansen et al., 1998; Wells et al., 1996; Wells et al., 1998). In chapter 2 of this dissertation, I found that subjects consumed ~550 additional calories during late-night hours and that these calories were higher in fat than calories consumed during morning, afternoon or evening hours. Interestingly, neurobehavioral deficits caused by sleep restriction are the most severe during late-night/early-morning hours (Goel et al., 2013). Thus, I became interested in examining whether or not late-night caloric intake contributes to the observed decline in neurobehavioral functioning. Although previous studies assessed the short-term effect of fasting on various neurobehavioral outcomes (Benau et al., 2014), no studies examined the effect of short-term fasting on late-night performance in the context of sleep restriction. In chapter 5 of this dissertation, I assessed sustained attention, working memory and cognitive throughput as well as subjective measures of sleepiness, stress and mood at 0200h during the fourth night following sleep restriction in subjects who were either fasted or fed from 2200h until bedtime.

ACKNOWLEDGMENTS

Portions of this introduction are from: Spaeth, A. M, Goel, N., and Dinges, D.F. (2012). Managing neurobehavioral capability when social expediency trumps biological imperatives. *Prog Brain Res,* 199, 377-398. The dissertation aims were originally thoughtout as the basis for a Ruth L. Kirschstein National Research Service Award (NRSA) which I was awarded by the National Institute of Aging in October, 2012.

CHAPTER 2: EFFECTS OF EXPERIMENTAL SLEEP RESTRICTION ON WEIGHT GAIN, CALORIC INTAKE, AND MEAL TIMING IN HEALTHY ADULTS

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This work originally appeared in *SLEEP* (2013) 36: 981-990.

ABSTRACT

Although population studies consistently show that short sleep duration associates with weight gain, no controlled laboratory studies have experimentally examined the effect of sleep restriction on weight gain in a large, diverse sample of men and women. The objective of this study was to examine sleep restriction's effects on weight gain, daily caloric intake, and meal timing. Two hundred twenty-five healthy adults aged 21–50 y (n = 198 sleep-restricted subjects/n = 31 with caloric intake data and n = 27 control subjects/n = 6 with caloric intake data) participated in the study and were randomized to an experimental condition including five consecutive nights of 4 h time in bed [TIB]/night, 0400h-0800h) or to a control condition (all nights 10 h TIB/night, 2200h-0800h). Sleep-restricted subjects gained more weight (0.97 ± (SD) 1.4 kg) than control subjects (0.11 ± (SD) 1.9 kg). Among sleep-restricted subjects, African Americans gained more weight than Caucasians and men gained more weight than women. Sleep-restricted subjects consumed excessive calories (130.0 ± (SD) 43.0% of daily caloric requirement) during days with a delayed bedtime (0400h) compared with control subjects who consumed adequate calories (100.6 ± (SD) 11.4%) during corresponding days. In sleep-restricted subjects, increased daily caloric intake was due to more meals and the consumption of 552.9 ± (SD) 265.8 additional calories between 2200h-0359h. The percentage of calories derived from fat was greater during late-night hours compared to daytime and evening hours. In the largest, most diverse healthy sample studied to date under controlled laboratory conditions, sleep restriction promoted weight gain. Chronically sleep-restricted adults with late bedtimes may be more susceptible to weight gain due to greater daily caloric intake and the consumption of calories during late-night hours.

INTRODUCTION

The 2004-2007 National Health Interview Survey revealed that approximately 28.3% of adults report sleeping 6 h or less per night and other studies have indicated that the prevalence of short sleepers (adults who report an average of ≤ 6 h of sleep within a 24-h period) has significantly increased in recent decades (Luckhaupt et al., 2010; Knutson et al., 2010). Reported associations between short sleep duration and energy homeostasis suggest the former may be a risk factor for a higher body mass index (BMI) (Cappuccio et al., 2008; Kobayashi et al., 2012; Appelhans et al., 2013) and prospective cohort studies have identified short sleep duration as a predictor of greater weight gain (Hasler et al., 2004; Chaput et al., 2008; Bo et al., 2011; Mozaffarian et al., 2011). This relationship may be stronger for men than for women (Watanabe et al., 2010; Meyer et al., 2012). However, no controlled laboratory studies have experimentally examined the effect of sleep restriction on weight gain in a large, diverse sample of men and women.

Laboratory studies have elucidated possible behavioral and physiological mechanisms underlying the relationship between sleep duration and weight gain. Spiegel and colleagues (2004b) found that subjects undergoing 2 nights of sleep restriction (4 h time in bed [TIB)/night) with controlled energy intake via an intravenous glucose infusion exhibited increased levels of ghrelin (an orexigenic hormone released from the stomach) and decreased levels of leptin (an anorexigenic hormone released from adipocytes). These neuroendocrine changes were accompanied by significant increases in self-reported ratings of hunger and appetite, specifically for foods with high carbohydrate content (Spiegel et al., 2004b). Other experiments have also shown that sleep loss increased ghrelin levels and decreased leptin in calorically-restricted humans (Spiegel et al., 2004a; Schmid et al., 2008; Nedeltcheva et al., 2010; Benedict et al., 2011). By contrast, in laboratory studies using ad libitum food access, which mimics a more natural environment, sleep loss was associated with either no change in ghrelin or leptin or an increase in leptin levels (Bosy-Westphal et al., 2008; Nedeltcheva et al., 2009b; Schmid et al., 2009; Omisade et al., 2010; Pejovic et al. 2010; Simpson et al., 2010). Such ad libitum

experiments, however, demonstrated that sleep loss was associated with increased caloric intake (Bosy-Westphal et al., 2008; Nedeltcheva et al., 2009b; Brondel et al., 2010; St-Onge et al., 2011). These investigations collectively indicate that humans compensate (or overcompensate) for sleep loss by increasing energy intake.

Sleep restriction also affects macronutrient intake and meal frequency. Adolescents who habitually slept less than 8 h per night consumed a higher proportion of calories from fat and a lower proportion of calories from carbohydrates and protein than those who slept 8 h or more per night (Weiss et al., 2010). In adults, sleep restriction has been associated with increased craving for foods high in carbohydrate content (Spiegel et al., 2004b) greater consumption of calories from carbohydrates (Nedeltcheva et al., 2009b) or fats (Brondel et al., 2010; St-Onge et al., 2011) and increased caloric intake derived from snacks (Nedeltcheva et al., 2009; Heath et al., 2012).

In addition to daily caloric intake, meal timing is an important contributor to weight gain (Arble et al., 2009; Garaulet et al., 2010). Baron and colleagues (2011) examined differences in sleep, eating, and weight between “normal sleepers” (sleep midpoint < 0530h) and “late sleepers” (sleep midpoint > 0530h) and found that late sleepers exhibited a shorter sleep duration, consumed more calories at dinner and after 2000h, consumed more fast food and full-calorie soda, and had a higher BMI compared to normal sleepers. In a group of healthy inpatients, individuals who ate during late-night/early morning (2300h-0500h) consumed more calories per day and gained more weight than non-nighttime eaters (Gluck et al., 2008). In an outpatient weight-loss effectiveness study, participants who were late eaters (lunch after 1500h) lost less total weight and displayed slower weight loss than early eaters (lunch before 1500h) (Garaulet et al., 2013). Animal studies have also shown a relationship between meal timing and weight gain: Circadian Locomotor Output Cycles Kaput (CLOCK) mutant mice exhibited an attenuated diurnal feeding rhythm and were hyperphagic and obese (Turek et al., 2005; Paschos et al., 2012). Moreover, mice exposed to light at night consumed more food during the light phase and had increased body mass compared with mice in a standard light/dark cycle, despite equivalent levels of caloric intake and total daily activity (Fonken et al., 2010).

The aforementioned lines of evidence suggest that sleep loss potentiates weight gain; however, few controlled experiments have manipulated sleep duration and objectively measured weight gain and ad libitum caloric intake. To address this deficiency, we experimentally tested the hypothesis that sleep restriction produces weight gain in a large sample of diverse healthy adults using a control condition. We also explored how sleep restriction affects possible contributors to weight gain and tested the hypotheses that sleep restriction increases daily caloric intake, increases meal frequency (snacking), and delays meal timing.

MATERIALS AND METHODS

Subjects

Two-hundred twenty-five healthy individuals, aged 21–50 y, were recruited in response to study advertisements. They reported habitual nightly sleep durations between 6.5 h and 8.5 h, habitual bedtimes between 2200h and 0000h, and habitual morning awakenings between 0600h and 0900h. They had no evidence of habitual napping, no sleep disturbances (i.e., no complaints of insomnia, daytime sleepiness, or other sleep-wake disturbances), and an absence of extreme morningness or extreme eveningness, as assessed by questionnaire (Smith et al., 1989). They were free of acute and chronic medical and psychological conditions, as established by interviews, clinical history, questionnaires, physical examinations, and blood (including a fasting blood glucose test) and urine tests. Subjects were monitored at home with actigraphy, sleep-wake diaries, and time-stamped call-ins to assess bedtime and wake time during the week prior to the in-laboratory phase and the week after the laboratory phase.

Subjects were nonsmokers and had a BMI ranging from 19–30. They did not participate in shift work, transmeridian travel, or irregular sleep/wake routines in the 60 days prior to the study. Sleep disorders were excluded by a night of laboratory polysomnography and oximetry measurements. Subjects were not permitted to use caffeine, alcohol, tobacco, and medications (except oral contraceptives) in the week before the laboratory experiment, as verified by blood

and urine screenings. The studies were approved by the Institutional Review Board of the University of Pennsylvania and all subjects were compensated for their participation.

Experimental Design

Subjects participated in one of five protocols in the Sleep and Chronobiology Laboratory at the Hospital of the University of Pennsylvania. Subjects were studied for 12, 14, or 18 consecutive days continuously with daily clinical checks of vital signs and symptoms by nurses (with an independent physician on call). Subjects were randomized as a group (n = 4 to 5 per group) to either the sleep restriction (SR) or control condition. In all five protocols, the SR condition consisted of two initial baseline nights of 10 h or 12 h TIB per night (2200h–0800h/1000h) followed by 5 nights of sleep restricted to 4 h TIB per night (0400h–0800h). A subset of subjects experienced 2 nights of recovery sleep (12 h TIB, 2200h-1000h) following sleep restriction. Sleep restriction consisting of 4 h TIB for 5 consecutive nights was selected because this degree of sleep loss produces cumulative neurobehavioral deficits in most healthy adults and is within the range of sleep loss that occurs as a result of lifestyle factors (Luckhaupt et al., 2010; Knuston et al., 2010; Van Dongen et al., 2003; Banks et al., 2009; Goel et al., 2009a).

The protocol days for the SR condition are labeled as follows throughout the manuscript: baseline (BL, the day following the first night of baseline sleep with a 2200h bedtime); extended wakefulness (EW, the day following the second night of baseline sleep with a 0400h bedtime); sleep restriction days 1–4 (SR1–4, days following SR with a 0400h bedtime); sleep restriction day 5 (SR5, the fifth day following sleep restriction with a 2200h bedtime) and recovery days 1–2 (R1–2, days following recovery sleep with a 2200h bedtime. The control condition involved the same procedures as the SR condition in each protocol, except that subjects were allowed 10 h TIB every night (2200h–0800h) during the in-laboratory stay.

During the in-laboratory phase of the study, subjects were not permitted to leave the laboratory. In both the SR and control conditions, subjects were ambulatory but were not allowed to exercise. Subjects were permitted to watch television, read, play video or board games, and

perform other sedentary activities between test bouts (which were completed while sitting at a computer).

Subjects wore a wrist actigraph throughout the in-laboratory protocol. On certain protocol days, subjects wore ambulatory electroencephalography (EEG) and electrocardiography (ECG) recording equipment for 24 h intervals. The light levels in the laboratory were held constant at < 50 lux during scheduled wakefulness and < 1 lux during scheduled sleep periods. Ambient temperature was maintained between 22°- 24°C. Subjects were behaviorally monitored by trained staff continuously throughout the protocol to ensure adherence.

Measures

Body Weight

Body weight was measured in N = 225 subjects (n = 198 sleep-restricted subjects and n = 27 control subjects). Of the control subjects (age 31.9 ± 8.4 y; BMI 25.0 ± 3.1 [mean ± standard deviation]), n = 12 (44%) were women and n = 17 (63%) were African American. Of the sleep-restricted subjects (age 31.3 ± 7.9 y; BMI 24.8 ± 3.3), n = 89 (45%) were women and n = 116 (59%) were African American. During a physical examination 6–7 days prior to the in-laboratory phase of the study, and upon admittance to and discharge from the in-laboratory protocol, nurses measured each subject's height and weight (while subjects wore minimal clothing and no shoes) at the Clinical and Translational Research Center (CTRC) at the Hospital of the University of Pennsylvania (HUP) using the same calibrated scale. All subjects were weighed during the physical examination between 1000h-1200h. The majority of subjects (n = 183, 81%) were weighed between 1300h-1500h during admittance and discharge from the in-laboratory phase of the study. Of the remaining subjects, n = 26 (12%) were weighed between 0900h-1200h during admittance and discharge, and n = 16 (7%) were weighed between 1400h-1600h during admittance and discharge. In all cases, subjects were not fasted during the weigh-in days and they had access to a restroom for optional voiding before being weighed.

Caloric Intake

In order to determine contributors to weight gain, caloric intake was measured in a subset of subjects (n = 31 sleep-restricted subjects: 52% women, 65% African American, age 34.4 ± 9.2 y, BMI 25.2 ± 3.7 and n = 6 control subjects: 33% women, 67% African American, age 34.0 ± 9.8 y, BMI 25.7 ± 3.1). All sleep-restricted subjects experienced 2 nights of baseline sleep followed by 5 nights of sleep restriction. Nineteen of the 31 sleep-restricted subjects experienced 2 nights of recovery sleep following SR. Control subjects experienced 10 h TIB per night for each night of the protocol.

Food/Drink Timing and Availability

Subjects selected their meals/snacks by choosing from various menu options, selecting additional food/drink available in the kitchen within the laboratory suite (which included a refrigerator, microwave, and toaster oven) and by making requests to the monitors and study coordinator. In order to ensure that subjects were provided sufficient time to eat each day, three 30- to 45-min opportunities were specified in the protocol during days with a 2200h bedtime (0900h, 1235h, and 1830h) and one additional 30-min opportunity to eat was specified in the protocol during days with a 0400h bedtime (0030h). In addition to these specified meal times, subjects were also allowed to consume food/drink at any time during the protocol other than when they were completing neurobehavioral tests. During a typical day, in addition to the meal times specified in the protocol, subjects could consume food/drink from 0945h-1000h, 1105h-1200h, 1310h-1400h, 1430h-1600h, 1630h-1645h, 1730h-1800h, 1920h-2000h, 2030h-2200h, 2230h-0000h, 0115h-0200h, and 0230h-0350h. Subjects were never told that they had to eat/drink and they were instructed to eat/drink whenever they wanted as long as it did not interfere with testing times. Subjects were also instructed that they could eat what they ordered or could select from other foods available in the laboratory kitchen and that they should eat as much (or as little) as they preferred. Subjects retrieved their own food/drink from the kitchen inside the laboratory suite whenever they wanted to eat/drink and could eat at a table in the common area or privately in their bedrooms.

Food/Drink Measurement

All food was weighed and recorded prior to being provided to subjects. To enhance the measurement accuracy of each food's weight, food was provided in individual containers (for example, a dinner consisting of chicken, peas, and rice was provided in three separate containers). Each day, a detailed description of the items and the amount consumed and intake time was recorded by trained monitors. Additionally, any food/drink that was left over after each meal was weighed and recorded. The intake data were entered into The Food Processor SQL program (ESHA Research, Salem, OR), a validated (Hise et al., 2002) professional nutrition analysis software and database program that provides components of food/drink intake including calories and macronutrients.

Statistical Analyses

Between-subjects analyses of variance (ANOVAs) (with study entry BMI, age, race, and gender as covariates) compared weight changes between control subjects and sleep-restricted subjects. Between-subjects ANOVAs (with study entry BMI and age as covariates) compared weight changes between gender and race groups. Repeated measures ANOVAs compared caloric intake, macronutrients, and meal timing across protocol days. Only 19 subjects were included in the analyses examining caloric intake during days following recovery sleep. Post hoc comparisons were performed with paired t-tests using the False Discovery Rate (Storey, 2002) to account for multiple comparisons in order to examine differences between BL, SR, and R days. Effect sizes were calculated using Cohen's d (Cohen, 1988) (small, d = 0.2; medium, d = 0.5; large, d = 0.8). Correlation analysis between weight gain and caloric intake was performed using Spearman's rho.

RESULTS

Weight Change

To examine if subjects were weight-stable at the start of the in-laboratory phase of the study, each subject's weight during admittance was compared with his/her weight during the physical examination (approximately a 1-w interval). The change in weight was not significantly different between control subjects (-0.04 ± (SD) 0.82 kg) and sleep-restricted subjects (0.09 ± 0.95 kg; $P > 0.4$) and neither group's change in weight was different from zero (all $P > 0.20$). During the in-laboratory protocol, sleep-restricted subjects (0.97 ± 1.43 kg) gained significantly more weight than control subjects (0.11 ± 1.85 kg; $F(1, 223) = 7.52$, $P = 0.007$, $d = 0.51$; **Figure 2.1A**). The change in weight during the protocol was not different from zero ($P > 0.71$) for control subjects but was significantly different from zero for sleep-restricted subjects ($P < 0.001$). The same pattern was observed when using weight change as a percentage of admittance body weight and BMI change as dependent variables. Sleep-restricted subjects gained a larger percentage of admittance body weight (1.4 ± 2.0%; $F(1, 223) = 7.40$, $P < 0.007$) and exhibited a greater increase in BMI (0.33 ± 0.49; $F(1, 223) = 8.42$, $P < 0.004$) than control subjects (percentage of admittance weight change: 0.2 ± 2.6%; BMI change: 0.03 ± 0.63). Sleep-restricted subjects whose caloric intake was monitored (n = 31) gained 0.52 ± 1.60 kg during the protocol and control subjects whose caloric intake was monitored (n = 6) lost 0.53 ± 1.16 kg during the protocol.

Among sleep-restricted subjects there were significant main effects for gender and race; men gained more weight than women ($F(1, 192) = 8.29$, $P = 0.004$, $d = 0.37$) and African Americans gained more weight than Caucasians ($F(1, 192) = 9.10$, $P = 0.003$, $d = 0.38$). African American men showed the most weight gain, Caucasian women showed the least weight gain, and Caucasian men and African American women showed intermediate weight gain (post hoc analyses illustrated in **Figure 2.1B**). There were no gender or race differences in weight change in control subjects (all $P > 0.10$). The same pattern was observed when using weight change as a percentage of admittance body weight and BMI change as dependent variables. Among sleep-

restricted subjects, African Americans gained a larger percentage of admittance body weight (1.7 ± 2.2%; F (1, 223) = 9.85, *P* = 0.002) and exhibited a greater increase in BMI (0.40 ± 0.52; F (1, 223) = 9.18, *P* = 0.003) than Caucasians (percentage of admittance weight change: 0.94 ± 1.8%; BMI change: 0.22 ± 0.42) and men gained a larger percentage of admittance body weight (1.6 ± 2.0%; F (1, 223) = 5.38, *P* = 0.02) and exhibited a greater increase in BMI (0.38 ± 0.50; F (1, 223) = 4.96, *P* = 0.03) than women (percentage of admittance weight change: 1.11 ± 1.96%; BMI change: 0.26 ± 0.47).

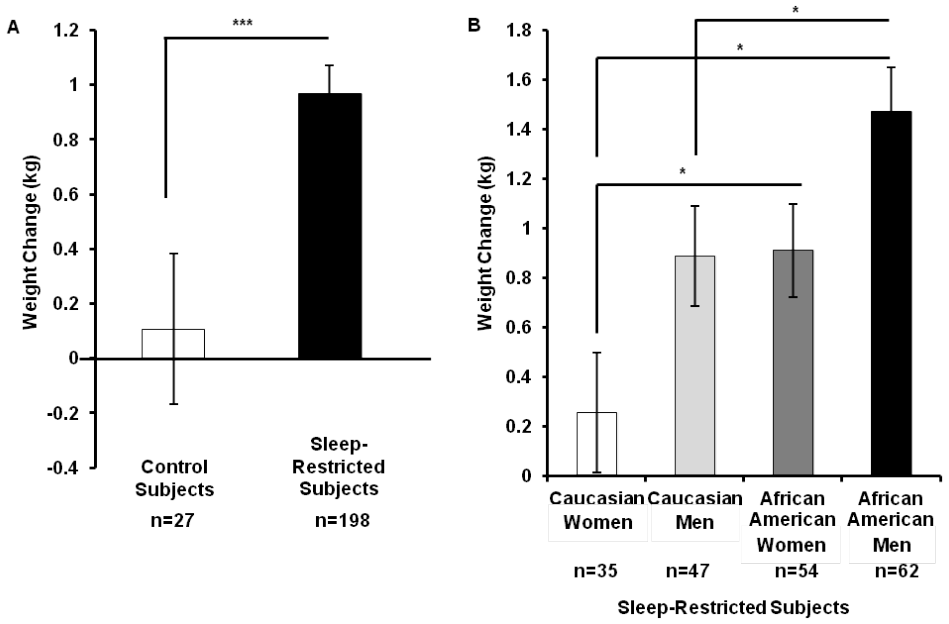

FIGURE 2.1: Effect of Sleep Loss on Weight Gain

(**A**) Subjects were healthy adults aged 21–50 y with a body mass index ranging between 19–30. Sleep-restricted subjects gained significantly more weight than control subjects (d = 0.51). (**B**) Among sleep-restricted subjects, African Americans gained more weight than Caucasians (d = 0.37) and men gained more weight than women (d = 0.38). Data expressed as mean ± SEM, * *P* < 0.05; ****P* < 0.001.

Caloric Intake

In control subjects, caloric intake did not vary significantly across protocol days (P = 0.09). By contrast, in sleep-restricted subjects, caloric intake varied significantly across BL and SR days ($F(6, 180) = 7.49$, $P < 0.001$) and across BL, SR, and R days ($F(8, 144) = 6.79$, $P < 0.001$). Subjects consumed more calories during days when bedtime was delayed until 0400h (EW, SR1-4; **Figure 2.2A**) compared to BL (post hoc analyses, ps < 0.05) and recovery days (post hoc analyses, all $P < 0.01$). On days when bedtime and hours spent awake were comparable (BL and SR5), caloric intake did not significantly differ (P = 0.79).

In order to compare caloric intake between sleep-restricted subjects and control subjects as well as to examine the relationship between caloric intake and weight change, caloric intake was calculated as a percentage: actual daily caloric intake / estimated daily caloric intake required for weight maintenance. Each subject's daily caloric intake required for weight maintenance was estimated using the Harris-Benedict equation (Harris and Benedict, 1918) for basal metabolic rate [(men = 66.4730 + (13.7516 * weight (kg)) + (5.0033 * height (cm)) – (6.755 * age (y)); women = 655.0955 + (9.5634 * weight (kg)) + (1.850 * height (cm)) – (4.676 * age (y))] multiplied by 1.4, which corresponds to a sedentary lifestyle representative of a laboratory study in which activity is limited (Black, 1996). Average caloric intake was not significantly different between control and sleep-restricted subjects during days with a 2200h bedtime (BL, SR5, R1-2; P = 0.58). However, sleep-restricted subjects consumed significantly more calories than control subjects during days when they had a delayed bedtime (EW, SR1-4; $F(1, 31.03) = 3.26$, d = 0.94, P = 0.003; **Figure 2.2B**). In sleep-restricted subjects, average caloric intake during days with a delayed bedtime (EW-SR4) was significantly positively correlated with weight change (Spearman's rho = 0.64, $P < 0.001$).

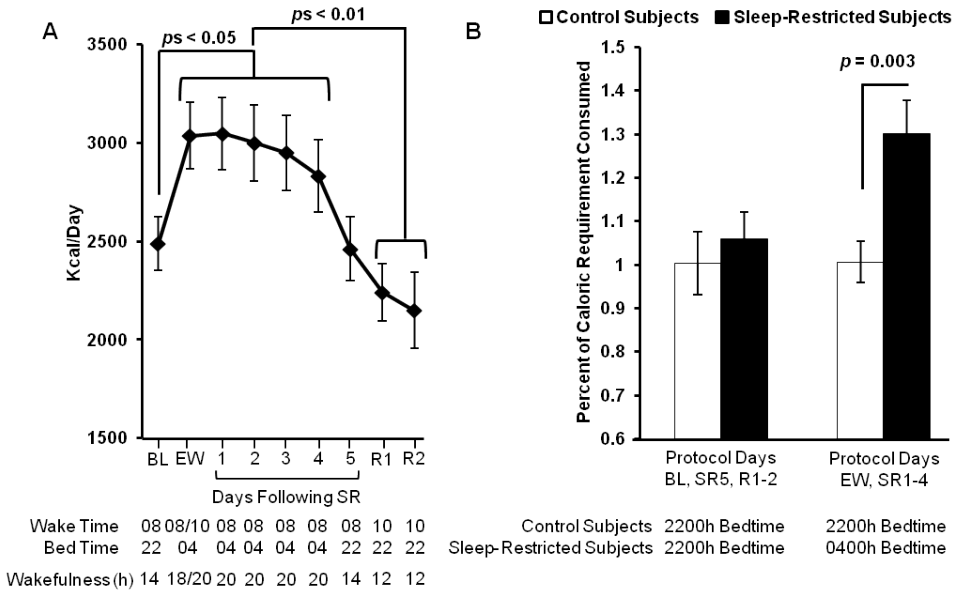

FIGURE 2.2: Caloric Intake by Protocol Day

(**A**) Sleep-restricted subjects consumed significantly more calories during days when bedtime was delayed to 0400h (EW-SR4) compared to days when bedtime was 2200h (BL, R1-2). Caloric intake did not differ between BL and SR5 (when waking hours were equivalent and bedtime was 2200h). Caloric intake did not differ between BL and each recovery day (R1-2). (**B**) Sleep-restricted subjects and control subjects did not differ in caloric intake during days when both groups had a 2200h bedtime (BL, SR5, R1-2). Sleep-restricted subjects consumed more calories than control subjects during days when they had a 0400h delayed bedtime (EW, SR1-4; d = 0.94). Data expressed as mean ± SEM.

Macronutrients

In sleep-restricted subjects, the number of grams consumed from each nutrient varied significantly across BL and SR days (protein: F (6, 180) = 5.74, $P < 0.001$; carbohydrates: F (6, 180) = 6.78, $P < 0.001$); fat: F (6, 180) = 4.70, $P < 0.001$) and across BL, SR, and R days (protein: F (8, 144) = 6.18, $P < 0.001$; carbohydrates: F (8, 144) = 5.23, $P < 0.001$); fat: F (8, 144) = 5.17, $P < 0.001$; **Table 2.1**). Consistent with increases in total caloric intake, sleep-restricted subjects consumed more grams of each nutrient during the first four delayed bedtime days (EW,

SR 1–3) compared to baseline (post hoc analyses, all $P < 0.05$) whereas on the fourth day following sleep restriction (SR4), when overall caloric intake remained greater than BL intake, only fat consumption was significantly greater than BL ($P < 0.05$; **Table 2.1**). Macronutrient consumption was not significantly different between BL and SR5 protocol days (all $P > 0.40$). Macronutrient consumption during each recovery day was not significantly different from BL or SR5 intake (all $P > 0.10$) but was lower than intake during delayed bedtime days (EW, SR1-4; post hoc analyses, all $P < 0.05$; **Table 2.1**).

To control for changes in total caloric intake across protocol days, the percentage of daily caloric intake derived from protein, carbohydrates, and fat were also calculated and compared (**Table 2.1**). There were no significant differences in the percentage of calories from protein, carbohydrate, or fat across all protocol days.

Meal Patterns

The US Department of Labor (2008) considers 30 min sufficient for a 'meal period'; therefore, meals were considered as discrete episodes if there was a minimum of 30 min between intake bouts. The total number of meals varied across BL and SR days ($F (6, 180) = 16.41$, $P < 0.001$) and across BL, SR, and R days ($F (8, 144) = 12.45$, $P < 0.001$; **Table 2.1**). Compared with baseline, subjects consumed more meals during days when bedtime was delayed (EW, SR1-4; post hoc analyses, all $P < 0.01$). The number of meals consumed during the fifth day following sleep restriction (SR5) did not differ from those consumed during BL ($P > 0.10$). Compared with each R day, subjects consumed more meals during days when bedtime was delayed (EW, SR1-4; post hoc analyses, all $P < 0.01$). The number of meals consumed during each R day did not differ from the number of meals consumed during BL and SR5 (all $P > 0.30$).

When examining average meal size, overall ANOVAs comparing meal size across BL and SR days ($F (6, 180) = 3.16$, $P = 0.006$) and across BL, SR, and R days ($F (8, 144) = 2.86$, $P < 0.01$) were significant (**Table 2.1**). However, post hoc analyses comparing EW, and SR1-5 protocol days to BL and post hoc analyses comparing BL, EW, and SR1-5 protocol days to each R day were not significant (all $P > 0.05$).

TABLE 2.1. Mean ± SD macronutrient content and meal patterns of caloric intake by protocol day

Macronutrients and meal patterns									
		Protein		Carbohydrates		Fats			
	Total calories	Grams	%	Grams	%	Grams	%	Meal number	Meal size (calories)
BL	2489.4 ± 759.2	85.2 ± 25.5	14.0	368.5 ± 118.8	59.2	79.9 ± 33.5	28.5	3.8 ± 0.9	644.1 ± 192.2
EW	3036.2 ± 949.9 [ab]	111.3 ± 34.2 [ab]	15.2	434.6 ± 145.0 [ab]	57.3	102.3 ± 38.1 [ab]	29.8	5.2 ± 0.9 [ab]	583.0 ± 207.6
SR1	3047.3 ± 1018.6 [ab]	101.1 ± 37.4 [ab]	13.3	446.4 ± 155.8 [ab]	58.7	103.0 ± 42.0 [ab]	30.1	4.6 ± 1.1 [ab]	670.9 ± 236.0
SR2	2999.3 ± 1083.8 [ab]	101.2± 39.5 [ab]	13.6	432.1 ± 161.3 [ab]	57.8	104.4 ± 44.5 [ab]	31.2	5.3 ± 1.7 [ab]	578.1 ± 209.4
SR3	2949.9 ± 1068.8 [ab]	101.2 ± 39.8 [ab]	13.9	436.7 ± 166.7 [ab]	59.2	95.3 ± 46.0 [ab]	28.9	5.2 ± 1.3 [ab]	573.9 ± 183.3
SR4	2833.0 ± 1016.5 [ab]	95.3 ± 43.4 [b]	13.4	402.4 ± 141.5 [b]	57.4	100.0 ± 43.9 [ab]	31.3	5.0 ± 1.5 [ab]	575.8 ± 224.7
SR5	2462.3 ± 894.9	84.0 ± 33.8	13.8	357.5 ± 131.6	58.7	82.66 ± 39.1	29.5	3.6 ± 0.8	685.2 ± 296.2
R1	2241.4 ± 628.4	69.0 ± 25.5	12.6	356.0 ± 97.7	63.8	66.4 ± 28.8	26.2	3.6 ± 0.8	624.4 ± 154.6
R2	2149.3 ± 839.0	73.0 ± 33.0	13.6	327.9 ± 138.1	61.2	65.4 ± 31.7	27.2	3.8 ± 1.1	576.6 ± 206.1

% is representative of total daily caloric intake

[a] Significantly different from BL; all $P < 0.05$.

[b] Significantly different from each Recovery Day (R1-2); all $P < 0.05$.

Meal Timing

Daily caloric intake was calculated for three time intervals: 0800h-1459h, 1500h-2159h and 2200h-0359h for BL and SR days. The first two time intervals were created by dividing the common waking hours across BL and SR protocol days into two equal 7-h intervals. The third time interval equaled the 6 h of wakefulness that occurred during delayed bedtime days (EW, SR1-SR4) but not during the baseline and SR5 protocol days. Recovery days were not included in these analyses due to a delayed wake time (1000h) that differed from the other protocol days.

Total caloric intake during 0800h-1459h was lower during days following SR (SR1-5) compared to days following BL sleep (BL and EW; F (1, 30) = 4.23, P = 0.047; **Figure 2.3A**); however, calories consumed during 1500h-2159h did not significantly differ between conditions (*P* = 0.096; **Figure 2.3A**). Caloric intake during 2200h-0359h varied across days (F (4, 120) = 3.48, *P* = 0.01): this overall difference was due to a significant reduction in calories consumed between the first and second nights with a delayed bedtime (EW and SR1; post hoc analyses, *P* < 0.05, **Table 2.2**). Across all nights with a delayed bedtime, subjects chose to consume calories during each hour of the late-night interval as follows: 2200h-2259h: n = 19 (61%), 2300h-2359h: n = 13 (42%), 0000h-0059h: n = 25 (81%), 0100h-0159h: n = 23 (74%), 0200h-0259h: n = 25 (81%), and 0300h-0359h: n = 13 (42%); thus, intake was not limited to only the specified meal time (0030), but occurred throughout the late-night interval. Notably, a majority of subjects voluntarily chose to consume calories during the late-night interval on each night: EW: n = 31 (100%), SR1: n = 26 (84%), SR2: n = 28 (90%), SR3: n = 29 (94%), SR4: n = 31 (100%).

The percentage of daily caloric intake during each of the three time intervals was calculated to control for total caloric intake changes across protocol days. The percentage of calories consumed during the first two time intervals differed significantly across BL and SR days (0800h-1459h: F (6, 180) = 11.21, *P* < 0.001; 1500h-2159h: F (6, 180) = 4.53, *P* < 0.001; **Table 2.2**). Subjects consumed a significantly lower percentage of calories from 0800h-1459h during delayed bedtime days compared to the day following baseline sleep (post hoc analyses, all *P* < 0.05), and a lower percentage of calories from 1500h-2159h during EW and SR4 protocol days

compared to the day following BL sleep (post hoc analyses all $P < 0.05$). The percentage of calories consumed during the late-night time interval varied across SR days (2200h-0359h: $F(4, 120) = 6.27$, $P < 0.001$; **Table 2.2**). The percentage of calories consumed from 2200h-0359h was greater during the first and fifth night of SR (EW and SR4) compared to the second and third nights of SR (SR1 and 2; post hoc analyses, all $P < 0.05$).

Macronutrients

The number of grams and the percentage of calories derived from protein, carbohydrate, and fat during each time interval were calculated and compared across protocol days. There were no significant differences in the macronutrient content (grams or percentage of calories) consumed during each time interval across protocol days (all $P > 0.05$). The macronutrient content during each time interval was averaged across all days and also compared. The macronutrient content of each time interval differed significantly (protein: ($F(2, 60) = 8.12$, $P = 0.001$; carbohydrates: $F(2, 60) = 3.48$, $P = 0.04$; fat: $F(2, 60) = 7.29$, $P = 0.001$; **Figure 2.3B**). The amount of calories derived from protein was significantly greater during 1500h-2159h and was significantly reduced during 2200h-0359h compared to the other two time intervals (all $P < 0.05$). Compared to the other two time intervals, the amount of calories derived from carbohydrates was significantly greater during 0800h-1459h (all $P < 0.05$) and the amount of calories derived from fat was significantly greater during 2200h-0359h (all $P < 0.05$).

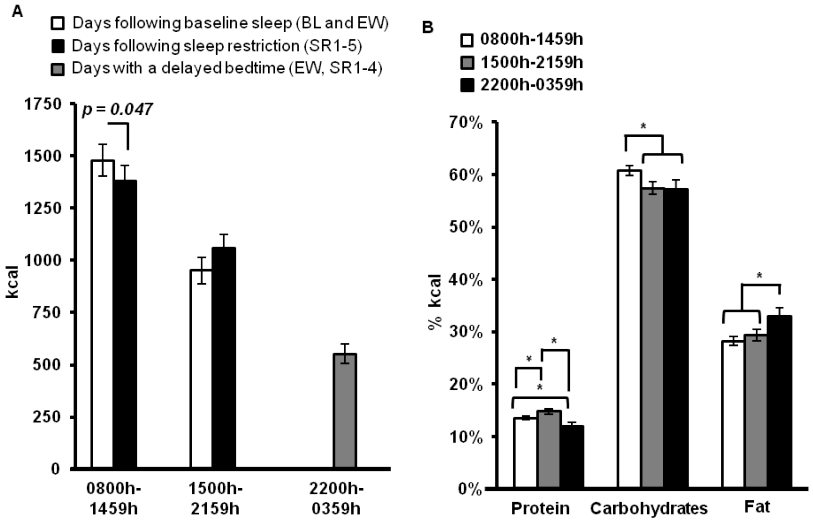

FIGURE 2.3: Effect of Sleep Loss on Meal Timing

(**A**) Subjects consumed significantly fewer calories from 0800h-1459h on days following sleep restriction (SR1-5) compared to days following baseline sleep (BL and EW). During days with a delayed bedtime (EW, SR1-4), subjects consumed an average of 552.9 calories from 2200h-0359h. (**B**) Compared to the other two time intervals, the amount of calories derived from protein was greater during 1500h-2159h and was reduced during 2200h-0359h, the amount of calories derived from carbohydrates was greater during 0800h-1459h, and the amount of calories derived from fat was greater during 2200h-0359h. Data expressed as mean ± SEM, * $P < 0.05$.

TABLE 2.2. Mean ± SD timing of caloric intake by protocol day

	Meal timing						
		0800h-1459h		1500h-2159h		2200h-0359h	
	Total Calories	Calories	%	Calories	%	Calories	%
BL	2489.4 ± 759.2	1475.3 ± 428.8	60.5	1014.1 ± 470.1	39.4	---	---
EW	3036.2 ± 949.9[a]	1481.4 ± 517.4	48.8[a]	887.8 ± 357.2	29.2[a]	666.9 ± 263.1	22.0
SR1	3047.3 ± 1018.6[a]	1450.9 ± 469.0	48.6[a]	1140.6 ± 452.2	37.8	455.7 ± 380.2[b]	13.6[c]
SR2	2999.3 ± 1083.84[a]	1374.4 ± 519.8	46.4[a]	1139.1 ± 453.5	39.0	485.8 ± 386.5	14.7[c]
SR3	2949.9 ± 1068.8[a]	1329.8 ± 463.1	46.7[a]	1075.2 ± 608.4	35.6	544.9 ± 326.8	17.7
SR4	2833.0 ± 1016.5[a]	1307.6 ± 487.0	47.6[a]	914.1 ± 437.3	32.2[a]	611.3 ± 392.3	20.2
SR5	2462.3 ± 894.9	1445.0 ± 552.9	59.6	1017.3 ± 473.5	40.4	---	---

% is representative of total daily caloric intake.

[a] Significantly different from BL; all $P < 0.05$.

[b] Significantly different from EW; $P < 0.05$.

[c] Significantly different from EW and SR4; all $P < 0.05$.

DISCUSSION

In the largest sample of healthy adults studied to date under controlled laboratory conditions, sleep-restricted subjects gained more weight than control subjects. Among sleep-restricted subjects, African Americans gained more weight than Caucasians and men gained more weight than women. Caloric intake during days with a delayed bedtime was positively associated with weight gain. Sleep-restricted subjects consumed an excessive amount of calories beyond daily caloric requirements during days with a delayed bedtime compared with control subjects who consumed an adequate amount of calories during corresponding days. Thus, increases in caloric intake in sleep-restricted subjects were not due to novelty of the laboratory setting or other environmental factors.

In sleep-restricted subjects, daily caloric intake was increased during days when their bedtime was delayed until 0400h (EW, SR1-4) compared to days when their bedtime was 2200h (BL, SR5, R1-2) and this increase was associated with greater intake of all three macronutrients and greater meal frequency. Compared to days following BL sleep (BL and EW), the amount of calories consumed from 0800h-1459h was reduced (by 96.8 calories), the amount of calories consumed from 1500h-2159h was not significantly changed, and 552.9 additional calories were consumed from 2200h-0359h during sleep restriction. Thus, the overall increase in caloric intake on days with a delayed bedtime was exclusively due to intake during the late-night period of additional wakefulness.

Our experimental weight gain findings support the relationship between short sleep duration and increased BMI observed in epidemiological studies (Hasler et al., 2004; Cappuccio et al., 2008; Chaput et al., 2008; Bo et al., 2011; Mozzaffarian et al., 2011; Kobayashi et al., 2012; Appelhans et al., 2013) and those studies indicating that men may be more susceptible to weight gain resulting from sleep loss (Watanabe et al., 2010; Meyer et al., 2012). Sleep-restricted African Americans were particularly vulnerable to weight gain—this finding is important considering African Americans are more likely to habitually sleep less than 6 h per night (Lauderdale et al., 2006; Knutson et al., 2010). We are currently examining the behavioral and physiological

mechanisms underlying differences in weight gain following sleep restriction between men and women and between African Americans and Caucasians.

Consistent with previous studies examining ad libitum food access during sleep restriction (Bosy-Westphal et al., 2008; Nedeltcheva et al., 2009b; St-Onge et al., 2011) our subjects increased caloric intake during days with a delayed bedtime. This caloric intake increase occurred during the EW day that did not follow sleep restriction but consisted of a 20-h day with a 0400h bedtime, and notably did not occur during the SR5 protocol day that followed sleep restriction but consisted of only 14 h of wakefulness and a 2200h bedtime. The increase in caloric intake on EW but not on SR5 suggests that a delayed bedtime and/or hours of wakefulness may be better predictors of caloric intake than sleep duration the preceding night. Future studies are needed to determine the amount of additional calories burned with a delayed bedtime to compare increased caloric intake and the increased energy expenditure required from extended wakefulness. Jung and colleagues (2011) measured energy expenditure in a total sleep deprivation paradigm and found that approximately 134 additional calories were needed for an 8-h extended wake time; however, their subjects were confined to bed rest, so this finding likely is an underestimation of energy requirements under normal activity conditions. The positive correlation between weight gain and caloric intake during sleep restriction in our study suggests that subjects consumed more calories than necessary to compensate for the additional energy requirement of extended wakefulness.

In the current study, meal times were specified in the protocol to ensure that subjects had adequate time to eat; however, subjects were told they could eat/drink whenever they were not testing. Subjects were never presented with food/drink or told that they had to eat at a specific time; rather, food and drinks were always available for them to retrieve from the laboratory kitchen. Based on this ad libitum design, we were able to examine meal patterns (the number of meals subjects consumed throughout the day and the size of each meal they consumed) as well as meal timing (when subjects chose to consume calories). We observed an increase in the number of meals consumed during days with a delayed bedtime without a change in average

meal size. This is an important finding because meal patterns are indicative of physiological mechanisms underlying caloric intake; the factors that control meal onset (meal number) are distinct from those that control meal termination (meal size) (Cummings and Overduin, 2007). Processes that promote meal termination and limit meal size include gastrointestinal signals such as gastric distention and cholecystokinin (Cummings and Overduin, 2007). Postprandial signals (which affect the interval to the next meal) and meal initiation signals (which influence the start of meals) regulate meal number (Cummings and Overduin, 2007). Based on our findings, it is more likely that sleep restriction and a delayed bedtime affected postprandial and meal initiation signals rather than satiation signals. For example, ghrelin, a meal initiation signal, increases meal number without affecting the size of meals and studies have shown that ghrelin levels are increased with sleep loss (Spiegel et al., 2004b; Nedeltcheva et al., 20010; Benedict et al., 2011). Future studies should focus on how ghrelin is modulated by extended wakefulness/sleep loss and examine other postprandial signals such as hypothalamic dopamine (Meguid et al., 2000) and amylin (Lutz, 2006).

Contrary to previous findings from a 14-day sleep restriction protocol (Nedeltcheva et al., 2009b) we did not observe an increase in carbohydrate intake during days with a delayed bedtime. The proportion of calories derived from protein, carbohydrates, and fat were consistent across all protocol days. Therefore, subjects did not over-consume a specific macronutrient at the expense of the other two macronutrients during sleep restriction. Our study only consisted of 5 nights of sleep restriction; therefore, we may have observed changes in macronutrient intake with a longer protocol. On protocol day SR4, subjects consumed significantly more grams of fat compared to the baseline day whereas grams of protein and carbohydrate consumed were not different between these 2 days. This increase in fat consumption is consistent with a sleep restriction experiment in adults which found an increase in fat consumption (St-Onge et al., 2011) and a study showing that adolescents who were short sleepers (less than 8 h per night) consumed more fat compared to their peers who slept 8 h or more per night (Weiss et al., 2010).

Recent research has highlighted the critical contribution of meal timing to weight regulation (Arble et al., 2009; Garaulet et al., 2010). We observed a shift in the timing of caloric intake during days with a delayed bedtime. Subjects consumed additional calories during the late-night period when they remained awake and then consumed fewer calories the morning following sleep restriction. Thus, the proportion of calories consumed was altered such that subjects consumed the majority of calories in early evening/late-night hours rather than in morning/early afternoon hours. Previous experiments have shown that mice gained more weight when consuming calories during a period when they were normally asleep compared to mice who were fed on a normal schedule, even when the same amount of calories were consumed (Fonken et al., 2010). Therefore, future studies should vary schedules of sleep restriction in humans to determine whether bedtime affects the timing of caloric intake and weight gain. In addition, studies examining brain activation and the neuroendocrine mechanisms underlying the relationship between sleep duration and energy balance, should focus on this late-night period, when additional calories are consumed.

We also observed an increase in the proportion of calories from fat during late-night hours; this increase may be particularly contributory to weight gain. Baron and colleagues (2013) found that the percentage of fat consumed after 2200h was associated with greater total caloric intake and a higher BMI among all individuals. In a laboratory controlled study, patients with night eating syndrome exhibited a delay in carbohydrate and fat intake compared to healthy control subjects (Goel et al., 2009b) and epidemiological studies have shown that patients with night eating syndrome are at greater risk for obesity (Tholin et al., 2009; Lundgren et al., 2006) and weight gain (Andersen et al., 2004; Gluck et al., 2008). Recent studies examining brain activation in the morning following sleep loss showed that neuronal activity in response to food stimuli was greater after restricted sleep compared to after habitual sleep (St-Onge et al., 2012a) and that total sleep deprivation was associated with increased activation of the right anterior cingulate cortex, an area involved in reward and anticipation, in response to food images (Benedict et al., 2012). Future studies focusing on sleep loss and brain activation should examine subjects during

the late-night period of extended wakefulness as this is the time when neuronal activity related to reward may be associated with increased fat consumption.

Limitations

Our study has several limitations. First, energy expenditure is an important factor that might contribute to weight gain. Subjects in our study were not allowed to exercise during the protocol; therefore, activity levels were limited. Because caloric intake was ad libitum, subjects did not fast during the protocol and therefore we could not assess resting metabolic rate, which may be affected by sleep loss. Second, although caloric intake was ad libitum, subjects were only allowed to consume food and drink provided by hospital and laboratory staff; foods that contained caffeine (including chocolate) were prohibited. In addition, although there were approximately 10 opportunities to eat during a typical protocol day, subjects were not allowed to eat/drink during neurobehavioral testing that occurred throughout the day. Therefore, subjects may have desired to eat certain foods that were unavailable to them or may have wanted to eat during certain times when they were not allowed to do so due to testing; both factors may have reduced total caloric intake and subsequent weight gain. Third, it should be noted that timing of caloric intake and voiding varied across subjects prior to weight measurements. Fourth, our subjects were healthy, were between the ages of 22–50 y, and had BMIs between the range of 19–30. The results may therefore not generalize to other groups, including obese individuals, adolescents or the elderly. Finally, the sample size of subjects with caloric intake information was too small to make comparisons between race and gender—thus, we cannot determine whether caloric intake underlies the race and gender weight gain differences.

Conclusions

Previous epidemiological studies indicate a relationship between short sleep duration and weight gain. The current study examined behavioral mediators of this relationship by objectively measuring weight, caloric intake, and meal timing in controlled laboratory protocols involving 5 nights of sleep restricted to 4 h TIB per night. Sleep-restricted subjects gained more weight compared to controls and showed significant gender and race differences in weight gain.

Chronically sleep-restricted subjects with late bedtimes may be more susceptible to weight gain and obesity due to overall greater caloric intake as well as increased consumption during late-night hours. Such caloric intake during late-night hours may be particularly contributory to weight gain as these calories appear to be greater in fat compared to calories consumed during morning, afternoon, and evening hours.

ACKNOWLEDGMENTS

This investigation was sponsored by the NIH grants R01 NR004281 (DFD) and F31 AG044102 (AMS); the Department of the Navy, Office of Naval Research Award No. N00014-11-1-0361 (NG); Clinical and Translational Research Center (CTRC) grant UL1TR000003; and National Space Biomedical Research Institute (NSBRI) through NASA award NCC 9-58 (DFD). The authors thank the subjects who participated in the experiments and the faculty and staff of the Division of Sleep and Chronobiology who helped acquire the data.

CHAPTER 3: GENDER AND RACE DIFFERENCES IN CALORIC INTAKE DURING SLEEP RESTRICTION IN HEALTHY ADULTS

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This work was accepted for publication in *The American Journal of Clinical Nutrition* April, 2014.

ABSTRACT

Evidence indicates men and African Americans may be more susceptible to weight gain resulting from sleep loss than women and Caucasians, respectively and increased daily caloric intake is a major behavioral mechanism underlying the relationship between sleep loss and weight gain. The objective of the current study was to assess gender and race differences in caloric intake, macronutrient intake and meal timing during sleep restriction. Forty-four healthy adults aged 21–50y (32.7±8.7y; n=21 women, n=16 Caucasians) completed an in-laboratory protocol including two consecutive baseline nights (10/12h time-in-bed [TIB]/night, 2200h-0800h/1000h) followed by five consecutive sleep restriction nights (4h TIB/night, 0400h-0800h). Caloric intake and meal timing data were collected during the two days following baseline sleep and during the first three days following sleep restriction. During sleep restriction, subjects increased daily caloric intake and fat intake, including obtaining more calories from condiments, desserts and salty snacks, and consumed 532.6 ± (SD) 295.6 calories during late-night hours (2200h-0359h). Men consumed more calories during baseline and sleep restriction, exhibited a greater percent increase in caloric intake during sleep restriction, and consumed a higher percentage of daily calories during late-night hours than women. African Americans and Caucasians did not significantly differ in increased caloric intake or meal timing during sleep restriction. However, during baseline and sleep restriction, African Americans consumed a higher percentage of daily calories from carbohydrates, a lower percentage of daily calories from protein and more daily calories from caffeine-free soda and juice than Caucasians. Men may be more susceptible to weight gain during sleep loss than women due to a larger increase in daily caloric intake, particularly during late-night hours. Greater intake of carbohydrates, specifically sugar-sweetened beverages, may underlie weight gain in African Americans.

INTRODUCTION

A growing body of epidemiological and laboratory evidence suggests that short sleep duration may be a risk factor for weight gain and obesity. Cross-sectional and prospective cohort studies have shown that short sleep duration is associated with a higher body mass index (BMI) (Ford et al., 2014; Moraes et al., 2013), an increased risk for obesity (Singh et al., 2005; Di Milia et al., 2013), and is a predictor of greater weight gain (Hasler et al., 2004; Chaput et al., 2008; Kobayashi et al., 2012). Two recent laboratory studies found that sleep restriction (sleep limited to 4-5h time-in-bed [TIB] per night for 5 consecutive nights) led to weight gain in healthy adults (Markwald et al., 2013; Spaeth et al., 2013).

Increased daily caloric intake is a major behavioral mechanism underlying the relationship between short sleep duration and weight gain. Sleep restriction leads to an increase in daily caloric intake (Bosy-Westphal et al., 2008; Brondel et al., 2010; St-Onge et al., 2011; Calvin et al., 2013; Markwald et al., 2013; Spaeth et al., 2013) and greater caloric consumption from snacking (Nedeltcheva et al., 2009b). It remains unclear whether this increased caloric intake is due to the overconsumption of a specific macronutrient: two studies found that sleep restriction increased carbohydrate consumption (Nedeltcheva et al., 2009b; Markwald et al., 2013) whereas another study reported increased fat consumption (St-Onge et al., 2011). Delayed meal timing has also been identified as a significant contributor to weight gain (Baron et al., 2011; Baron et al., 2013; Garaulet et al., 2013; Allison et al., 2014). Sleep restriction promotes the consumption of additional calories during evening and late-night hours (Markwald et al., 2013; Spaeth et al., 2013).

Several epidemiological studies have found a stronger association between shorter sleep duration and higher BMI in men than women (Ko et al., 2007; Meyer et al., 2012; Yang et al., 2013). This pattern has also been observed in adolescents (Knutson, 2005; Araujo et al., 2012) and in children (Shi et al., 2010; Tatone-Tokuda et al., 2012). Using self-report surveys, two recent epidemiological studies found that sleep duration was inversely associated with BMI in men only, whereas poor sleep quality was positively associated with BMI in women only (Meyer

et al., 2012; Yang et al., 2013). In a prospective cohort study, short sleep duration (<5 and 5-6h per night) was associated with weight gain and the development of obesity at one-year follow-up in men but not in women (Watanabe et al., 2010). Finally, we found that men gained more weight than women during a controlled sleep restriction experiment (Spaeth et al., 2013).

Race differences also exist in the relationship between sleep duration and weight. Although African Americans are more likely to be shorter sleepers than Caucasians (Singh et al., 2005; Hale and Do, 2007), only two epidemiological studies have focused on such differences in the relationship between sleep duration and BMI. Both found that the association between short sleep duration and increased odds for obesity was stronger in African Americans than Caucasians (Donat et al., 2013; Grandner et al., 2014). In a controlled sleep restriction experiment, we found that African Americans gained more weight than Caucasians (Spaeth et al., 2013).

Collectively, these lines of evidence suggest that sleep loss potentiates weight gain by promoting an increase in daily caloric intake and that there are gender and race differences in the propensity for such weight gain. However, it is unknown whether gender or race differences exist in the increased caloric intake response to sleep restriction. To address this deficiency, we experimentally tested the hypothesis that men and African Americans will exhibit a greater increase in caloric intake during sleep restriction than women and Caucasians, respectively. We also explored gender and race differences in macronutrient intake and meal timing.

MATERIALS AND METHODS

Subjects

Healthy individuals, aged 21–50 y, were recruited in response to study advertisements. They reported habitual nightly sleep durations between 6.5h and 8.5h, habitual bedtimes between 2200h and 0000h, and habitual morning awakenings between 0600h and 0900h; these reports were confirmed objectively using actigraphy. They had no evidence of habitual napping, no sleep disturbances (i.e., no complaints of insomnia, daytime sleepiness, or other sleep-wake

disturbances), and neither extreme morningness nor extreme eveningness, as assessed by questionnaire (Smith et al., 1989). Subjects were free of acute and chronic medical and psychological conditions, as established by interviews, clinical history, questionnaires, physical examinations, and blood (including a fasting blood glucose test) and urine tests. They were nonsmokers and did not participate in shift work, transmeridian travel, or irregular sleep/wake routines in the 60 days prior to the study. Enrolled subjects were monitored at home with actigraphy, sleep-wake diaries, and time-stamped call-ins to assess bedtime and waketime during the week prior to the in-laboratory phase and the week after the laboratory phase. They were not permitted to use caffeine, alcohol, tobacco, and medications (except oral contraceptives) in the week before the laboratory experiment, as verified by urine screenings. Sleep disorders were excluded by a night of laboratory polysomnography and oximetry measurements.

The research protocols were approved by the Institutional Review Board of the University of Pennsylvania. All subjects provided written informed consent before enrollment and were compensated for their participation.

Experimental Design

Subjects participated in one of two protocols in the Sleep and Chronobiology Laboratory at the Hospital of the University of Pennsylvania and were studied for 14 or 18 consecutive days continuously with daily clinical checks of vital signs and symptoms by nurses (with an independent physician on call). Both protocols consisted of two initial baseline nights of 10h or 12h TIB per night (2200h–0800h/1000h) followed by five nights of sleep restricted to 4h TIB per night (0400h–0800h). This dose of sleep restriction was selected because it produces cumulative neurobehavioral deficits in most healthy adults (Van Dongen et al., 2003) and is within the range of sleep loss that occurs as a result of lifestyle factors (Luckhaupt et al., 2010).

During the in-laboratory phase of the study, subjects were not permitted to leave the laboratory. Subjects were ambulatory and were permitted to watch television, read, play video or board games, and perform other sedentary activities between test bouts (which were completed

while sitting at a computer) but were not allowed to exercise. Subjects wore a wrist actigraph throughout the in-laboratory protocol. On certain protocol days, subjects wore ambulatory electroencephalography (EEG) and electrocardiography (ECG) recording equipment for 24-h intervals. The light levels in the laboratory were held constant at < 50 lux during scheduled wakefulness and < 1 lux during scheduled sleep periods. Ambient temperature was maintained between 22°- 24°C. Subjects were behaviorally monitored by trained staff continuously throughout the protocol to ensure adherence.

Measures

Subjects selected their meals/snacks by choosing from various menu options, selecting additional food/drink available in the kitchen within the laboratory suite (which included a refrigerator, microwave, and toaster oven) and by making requests to the study staff. To ensure subjects were provided sufficient time to eat each day, three 30- to 45-min eating opportunities were specified in the protocol during days with a 2200h bedtime (0900h, 1235h, and 1830h) and one additional 30-min opportunity to eat was specified in the protocol during days with a 0400h bedtime (0030h). In addition to these specified meal times, subjects were allowed to consume food/drink at any time during the protocol other than when they were completing neurobehavioral tests or sleeping. During a typical day, in addition to the meal times specified in the protocol, subjects could consume food/drink from 0945-1000h, 1105h-1200h, 1310h-1400h, 1430h-1600h, 1630h-1645h, 1730h-1800h, 1920h-2000h, 2030h-2200h, 2230h-0000h, 0115h-0200h, and 0230h-0350h. Subjects could eat what they pre-ordered through menus or could select from other foods available in the laboratory kitchen and could eat as much (or as little) as they wanted. Subjects retrieved their own food/drink from the laboratory kitchen when they wanted to eat/drink and could eat at a table in the common area or privately in their bedrooms.

All food was weighed and recorded prior to being provided to the subjects. To enhance the measurement accuracy of each food item's weight, items were served in individual containers. Each day, a detailed description of the items and the amount consumed and intake time were recorded by trained monitors. Additionally, any food/drink that was left over after each

meal was weighed and recorded. The intake data were entered into The Food Processor SQL program (ESHA Research, Salem, OR), a validated (Hise et al., 2002) professional nutrition analysis software and database program that provides components of food/drink intake including calories and macronutrients.

Caloric intake from waketime (0800h/1000h) until 2200h during the two days following baseline sleep was averaged and defined as baseline (BL) caloric intake. Caloric intake from waketime (0800h) until 0359h during the first three days following sleep restriction was averaged and defined as sleep restriction (SR) caloric intake. Caloric intake was also measured during the fourth and fifth days following sleep restriction; however, these data were not included in the present analyses because some of the subjects (n=18) were fasted during the fourth day of sleep restriction as part of a separate experiment.

Statistical Analyses

Repeated measures analyses of variance (ANOVAs) compared caloric intake, macronutrient intake, and meal timing between BL and SR in all subjects. Mixed-model ANOVAs, with age entered as a covariate, BL versus SR as the repeated-measures variable and race and gender as between-subjects factors compared gender and race differences in the change in daily caloric intake (including calories consumed from specific food/drink categories), macronutrient intake and meal timing. Between-subjects ANOVAs, with age entered as a covariate, examined differences in caloric intake during BL and SR, increased caloric intake (caloric intake during SR – caloric intake during BL), macronutrient intake (% calories derived from protein, carbohydrates and fat during BL and SR), food/drink categories, and late-night eating (% of daily calories consumed between 2200h-0359h during SR) between gender and race groups. Effect sizes were calculated using Cohen's d (Cohen, 1988). The False Discovery Rate (Storey, 2002) was used when examining differences related to food/drink categories in order to account for multiple comparisons.

RESULTS

Subject Characteristics

Forty-four of the N=47 enrolled subjects completed the study. The three non-completers were either withdrawn due to protocol non-compliance (n=1) or withdrew due to health or personal issues unrelated to the protocol (n=2). Men (n=23, 1.78 ± 0.08 m; 82.4 ± 14.62 kg) were taller and weighed more than women (n=21, 1.63 ± 0.07 m; 65.30 ± 9.86 kg; ps < 0.001). Men and women did not differ in age (*P* = 0.28), BMI (*P* = 0.29), chronotype (*P* = 0.24) or in the percentage of African Americans and Caucasians (*P* = 0.82; **Table 3.1**). African Americans (n=28) and Caucasians (n=16) did not differ in height (*P* = 0.10), weight (*P* = 0.34), age (*P* = 0.14), BMI (*P* = 0.96), chronotype (*P* = 0.52) or in the percentage of men and women (*P* = 0.82; **Table 3.1**). Sleep duration and timing during the week prior to the in-laboratory study were assessed using wrist actigraphy; there were no gender or race differences in pre-study sleep duration, onset, offset or midpoint (all *P* > 0.10; **Table 3.1**).

TABLE 3.1. Subject characteristics (Mean ± SD)

	N	Age	BMI	% Men (n)	% African American (n)	Chron-otype[a]	Sleep Duration[b] (h)	Sleep Midpoint[b] (time ± min)
All Subjects	44	32.7 ± 8.7	25.2 ± 3.5	52.2	63.6	40.75 ± 5.82	8.04 ± 0.34	03:39 ± 46.8
Men	23	34.1 ± 7.9	25.7 ± 3.3	---	65.2 (15)	41.65 ± 5.76	8.02 ± 0.55	03:24± 41.4
Women	21	31.2 ± 9.6	24.6 ± 3.7	---	61.9 (13)	39.76 ± 5.87	8.06 ± 0.35	03:54 ± 48.6
African Americans	28	31.3 ± 8.0	25.2 ± 3.2	53.6 (15)	---	41.32 ± 5.50	7.97 ± 0.48	03:37 ± 49.2
Caucasians	16	35.3 ± 9.6	25.2 ± 4.1	50.0 (8)	---	39.75 ± 6.40	8.16 ± 0.41	03:40 ± 43.8

There were no significant differences between gender or race groups, all *P* > 0.10

[a]Morningness-Eveningness Composite Scale

[b]Sleep duration and sleep midpoint were measured using wrist actigraphy supplemented by sleep diary for one week prior to study entry.

Caloric Intake

Caloric intake was significantly greater during SR than during BL ($F(1, 43) = 54.94$, $P < 0.001$). Caloric intake was also calculated as a percentage: actual daily caloric intake / estimated daily caloric intake required for weight maintenance. Each subject's daily caloric intake required for weight maintenance was estimated using the Harris-Benedict equation (Harris and Benedict, 1918) for basal metabolic rate multiplied by 1.4, which corresponds to a sedentary lifestyle representative of a laboratory study in which activity is limited (Black, 1996). Subjects consumed 109.28 ± (SD) 29.54% of caloric need during BL and 133.04 ± (SD) 36.65% of caloric need during SR. During both conditions, subjects consumed significantly more calories than the amount needed to fulfill 100% of this estimation of caloric need (BL: $t(43) = 2.08$, $P = 0.043$; SR: $t(43) = 5.98$, $P < 0.001$); however, intake (calculated as a percentage of caloric need) during SR was significantly greater than during BL ($t(43) = 7.57$, $P < 0.001$).

A mixed-model ANOVA revealed a significant gender interaction ($F(1, 39) = 6.99$, $P = 0.012$): men consumed more calories during BL ($F(1, 39) = 4.60$, $P = 0.038$) and SR ($F(1, 39) = 10.15$, $P = 0.003$) and exhibited a greater increase in caloric intake than women during sleep restriction (SR – BL; $F(1, 39) = 6.99$, $P = 0.012$, $d = 0.92$; **Figure 3.1A**). Controlling for differences in BL caloric intake, men also exhibited a greater percent increase in caloric intake than women during sleep loss (SR / BL; men = 28.5%, women = 16.9%; $F(1, 42) = 4.27$, $P = 0.045$, $d = 0.62$). By contrast, the race interaction was not significant ($F(1, 39) = 0.047$, $P = 0.83$). Caloric intake during BL ($P = 0.45$) and SR ($P = 0.64$), and the change in caloric intake (SR – BL; $P = 0.83$) did not differ between African Americans and Caucasians (**Figure 3.1B**).

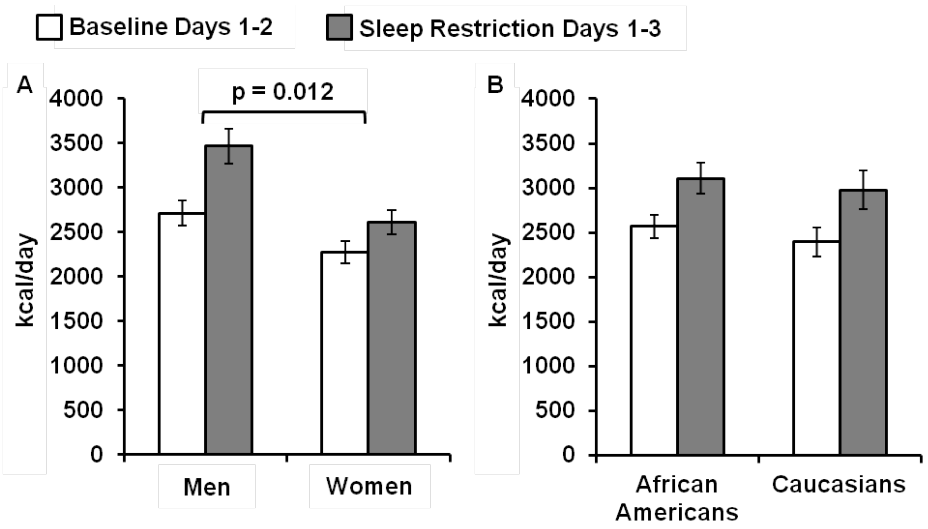

FIGURE 3.1: Daily Caloric Intake during Baseline and Sleep Restriction

Subjects consumed more calories during sleep restriction than during baseline ($p < 0.001$). (**A**) Daily caloric intake showed a significant gender interaction ($P = 0.012$), whereby men consumed more calories during both baseline ($P = 0.038$) and sleep restriction ($P = 0.003$) and exhibited a greater increase in caloric intake during sleep loss (SR-BL; $P = 0.012$, $d = 0.92$). Controlling for differences in baseline caloric intake, men also exhibited a greater percent increase in caloric intake than women (SR/BL; 28.5% vs. 16.9%; $P = 0.045$, $d = 0.62$). (**B**) The race interaction for daily caloric intake was not significant; African Americans and Caucasians did not differ in caloric intake during baseline or sleep restriction and exhibited a similar increase (SR-BL) in caloric intake during sleep restriction (all $P > 0.40$). Data expressed as mean ± SEM.

Macronutrient Intake

The percentage of calories derived from protein was significantly lower ($F(1, 43) = 14.73$, $P < 0.001$) and the percentage of calories derived from fat was significantly higher ($F(1, 43) = 5.50$, $P = 0.024$) during SR than BL (**Table 3.2**). The percentage of calories derived from carbohydrates did not differ between BL and SR ($P = 0.97$). There were no significant gender interactions for the percentage of calories derived from protein, carbohydrates and fat (all $P > 0.63$) from BL to SR and there were no significant differences in macronutrient intake between

men and women during BL or SR (all $P > 0.70$). There was a significant race interaction for the percentage of calories derived from protein ($F(1, 39) = 5.58$, $P = 0.023$) but not for the percentage of calories derived from carbohydrates or fat (all $P > 0.41$). During BL, African Americans consumed a significantly higher percentage of calories from carbohydrates ($F(1, 39) = 5.06$, $P = 0.03$, $d = 0.79$) and a lower percentage of calories from protein ($F(1, 39) = 25.96$, $P < 0.001$, $d = 1.71$) than Caucasians. During SR, African Americans also consumed a lower percentage of calories from protein ($F(1, 39) = 4.12$, $P = 0.049$, $d = 0.72$) and tended to consume a higher percentage of calories from carbohydrates than Caucasians; however this did not reach significance ($F(1, 39) = 3.21$, $P = 0.08$, $d = 0.69$; **Table 3.2**). The percentage of calories from fat did not differ between races during BL or SR (all $P > 0.42$).

TABLE 3.2. Mean ± SD macronutrient intake during baseline and sleep restriction

	Baseline Days 1-2			Sleep Restriction Days 1-3		
	Protein (%)	Carbo-hydrates (%)	Fat (%)	Protein (%)	Carbo-hydrates (%)	Fat (%)
All Subjects	14.9 ± 0.03	58.0 ± 0.06	28.9 ± 0.05	13.3 ± 0.02[a]	58.0 ± 0.04	30.6 ± 0.05[a]
Men	15.1 ± 0.02	58.1 ± 0.06	28.6 ± 0.05	13.3 ± 0.02	58.0 ± 0.05	30.5 ± 0.05
Women	14.6 ± 0.03	57.8 ± 0.06	29.2 ± 0.05	13.3 ± 0.02	58.0 ± 0.04	30.6 ± 0.04
African Americans	13.5 ± 0.02[b]	59.6 ± 0.05[b]	28.3 ± 0.05	12.8 ± 0.02[b]	59.0 ± 0.04	30.1 ± 0.05
Caucasians	17.2 ± 0.02	55.3 ± 0.06	29.9 ± 0.06	14.3 ± 0.02	56.1 ± 0.04	31.3 ± 0.04

Macronutrients presented as calories derived from protein, carbohydrates or fat divided by daily caloric intake.

[a] Significantly different from baseline, all $P < 0.05$

[b] Significantly different from Caucasians, all $P < 0.05$

To determine the source of the observed macronutrient intake changes, we assessed the calories consumed from the following food/drink categories during BL and SR: 1) meat, eggs and fish; 2) fruit, vegetables and salad; 3) bread, cereal, plain rice and pasta; 4) condiments (ketchup, mustard, mayonnaise, peanut butter, syrup, and jelly); 5) desserts; 6) salty snacks (chips, pretzels, crackers and popcorn); 7) caffeine-free soda and juice; and 8) milk (Beebe et al., 2013). During SR, subjects consumed more calories from bread, cereal, plain rice and pasta ($F(1, 43) = 5.21$, $P = 0.028$), condiments ($F(1, 43) = 7.41$, $P = 0.009$), desserts ($F(1, 43) = 13.36$, $P = 0.001$), salty snacks ($F(1, 43) = 8.29$, $P = 0.006$) and caffeine-free soda and juice ($F(1, 43) = 14.93$, $P < 0.001$) than during BL (all comparisons remained significant after the False Discovery Rate correction). Calories consumed from meat, eggs and fish ($P = 0.086$), fruit, vegetables and salad ($P = 0.66$), and milk ($P = 0.061$) did not differ between BL and SR (**Table 3.3**).

TABLE 3.3. Mean ± SD caloric intake by food/drink category

Food/Drink Category	Baseline Days 1-2 (kcal)	Sleep Restriction Days 1-3 (kcal)
Meat, eggs and fish	381.55 ± 128.92	419.77 ± 149.36
Fruit, vegetables and salad	177.65 ± 149.61	170.66 ± 138.02
Bread, cereal, plain rice and pasta	572.27 ± 192.71	659.51 ± 259.88[a]
Condiments	231.69 ± 139.55	276.44 ± 181.81[a]
Desserts	343.38 ± 286.83	544.34 ± 284.23[a]
Chips, pretzels, crackers and popcorn	78.88 ± 123.72	150.91 ± 202.57[a]
Caffeine-free soda and juice	272.64 ± 160.91	346.18 ± 219.36[a]
Milk	84.16 ± 98.45	119.78 ± 152.89

[a] Significantly higher than baseline, all $P < 0.05$

Consistent with the null findings related to gender differences in macronutrient intake, the gender interactions were not significant for each food/drink category (all $P > 0.15$). Men did consume more calories from bread, cereal, pasta and rice than women during SR ($F(1, 39) = 10.0$, $P = 0.003$) but there were no other significant gender differences for caloric intake from food/drink categories during either BL or SR (all $P > 0.05$). By contrast, there was a significant race interaction for fruits, vegetables and salad ($F(1, 39) = 9.48$, $P = 0.004$) and a trend for meats, eggs and fish ($F(1, 39) = 4.38$, $P = 0.043$; not significant after the False Discovery Rate correction). African Americans consumed fewer calories from fruits, vegetables and salad during BL ($F(1, 39) = 11.08$, $P = 0.002$, d = 0.88) but did not differ from Caucasians during SR ($P = 0.29$). In addition, during both BL and SR, African Americans consumed more calories from caffeine-free soda and juice than Caucasians (BL: $F(1, 39) = 13.09$, $P = 0.001$, d = 1.18; SR: $F(1, 39) = 12.05$, $P = 0.001$, d = 1.19). Calories consumed from the other food/drink categories did not differ between African Americans and Caucasians during either BL or SR (all $P > 0.05$).

Meal Timing

Daily caloric intake was calculated for three time intervals: 0800h-1459h, 1500h-2159h and 2200h-0359h; the first two time intervals were created by dividing the common waking hours from BL and SR protocol days into two equal 7-h intervals and the third time interval equaled the 6-h of wakefulness that occurred only during SR (Spaeth et al., 2013). Caloric intake during each time interval was also calculated as a percentage of daily caloric intake.

During SR, subjects consumed fewer calories ($F(1, 43) = 7.72$, $P = 0.008$) and a lower percentage of daily caloric intake ($F(1, 43) = 84.64$, $P < 0.001$) from 0800h-1459h than during BL. Also during SR, subjects consumed more calories from 1500h-2159h ($F(1, 43) = 12.12$, $P = 0.001$) than during BL; however the percentage of daily caloric intake consumed during this time period did not differ between conditions ($P = 0.32$). During the late-night hours of additional wakefulness during SR (2200h-0359h), subjects consumed 532.6 ± 295.6 (SD) calories which accounted for 16.37 ± 6.60 % of daily caloric intake.

There were no significant gender interactions for calories consumed between 0800h-1459h and 1500h-2159h (all $P > 0.29$) or for the percentage of daily caloric intake consumed during these time intervals (all $P > 0.34$) from BL to SR. During BL, men and women did not differ in caloric intake between 0800h-1459h ($P = 0.19$); however, men consumed more calories between 1500h-2159h ($F (1, 39) = 5.75$, $P = 0.021$, $d = 0.66$; **Figure 3.2A**). During SR, men consumed more calories than women between 0800h-1459h ($F (1, 39) = 4.39$, $P = 0.043$, $d = 0.78$), 1500h-2159h ($F (1, 39) = 7.39$, $P = 0.010$, $d = 0.87$) and 2200h-0359h ($F (1, 39) = 13.64$, $P = 0.001$, $d = 1.16$; **Figure 3.2B**). Controlling for gender differences in daily caloric intake, men did not differ from women in the percentage of daily caloric intake consumed from 0800h-1459h or 1500-2159h during BL (all $P > 0.21$). However, relative to women, men consumed a lower percentage of daily calories from 0800h-1459h ($F (1, 39) = 4.26$, $P = 0.046$, $d = 0.52$), a similar percentage of daily calories from 1500h-2159h ($P = 0.68$) and a higher percentage of daily calories from 2200h-0359h ($F (1, 39) = 7.89$, $P = 0.008$, $d = 0.78$; **Figure 3.2C**) during SR.

There were no significant race interactions for calories consumed between 0800h-1459h and 1500h-2159h (all $P > 0.63$) or for the percentage of daily caloric intake consumed during these time intervals (all $P > 0.59$) from BL to SR. African Americans and Caucasians did not differ in the amount of calories consumed during each time interval during BL or SR (all $P > 0.40$). Moreover, African Americans and Caucasians did not significantly differ in the percentage of daily caloric intake from 0800h-1459h or from 1500h-2159h during BL or SR (all $P > 0.13$). African Americans tended to consume a lower percentage of daily caloric intake from 2200h-0359h than Caucasians; however this did not reach significance ($F (1, 39) = 3.47$, $P = 0.07$).

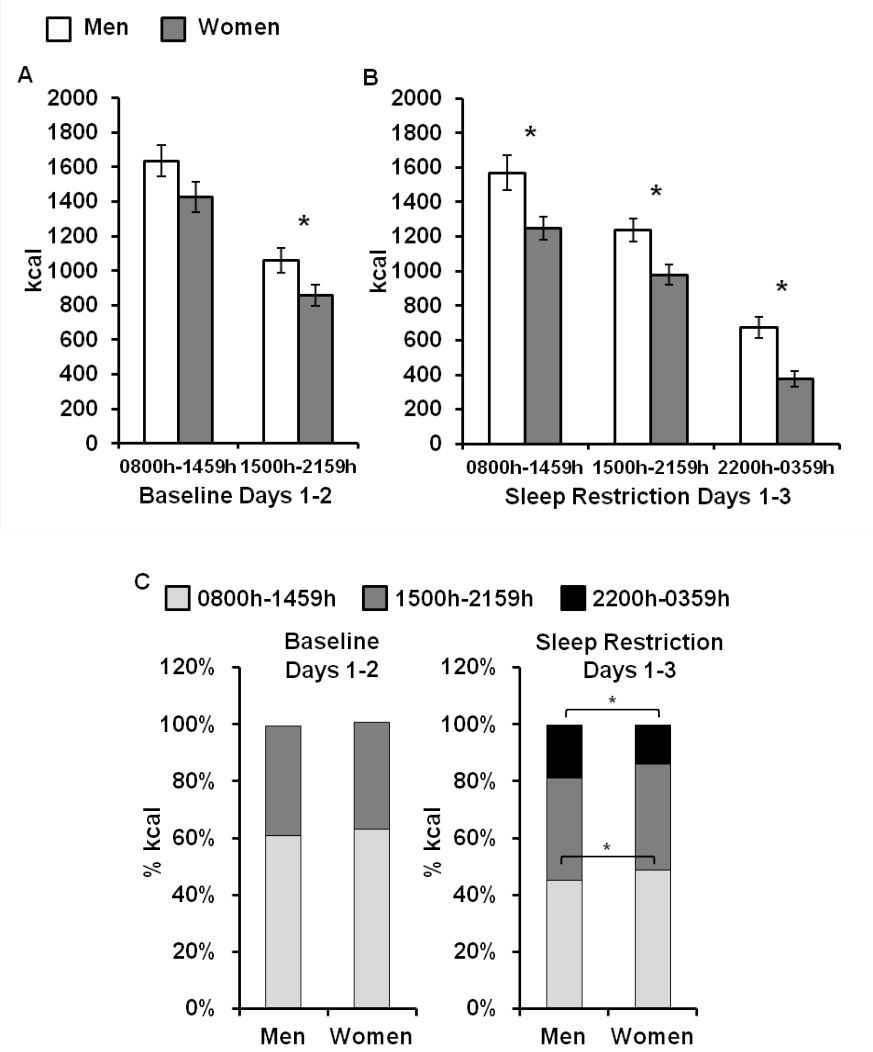

FIGURE 3.2: Meal Timing during Baseline and Sleep Restriction

(**A**) During baseline, men and women did not differ in caloric intake between 0800h-1459h; however, men consumed more calories than women between 1500h-2159h (*P* = 0.021, d = 0.66). (**B**) During sleep restriction, men consumed more calories than women between 0800h-1459h (*P* = 0.043, d = 0.78), 1500h-2159h (*P* = 0.010, d = 0.87) and 2200h-0359h (*P* = 0.001, d = 1.16). (**C**) Men did not differ from women in the percentage of daily caloric intake consumed from 0800h-1459h or 1500-2159h during baseline. However, relative to women, men consumed a lower percentage of daily calories from 0800h-1459 (*P* = 0.046, d = 0.52), a similar percentage of daily calories from 1500h-2159h, and a higher percentage of daily calories from 2200h-0359h (*P* = 0.008, d = 0.78) during SR.* *P* < 0.05, data expressed as mean ± SEM.

DISCUSSION

In the first study to systematically examine gender and race differences in caloric intake during experimental sleep loss, we found that men exhibited a greater increase in daily caloric intake during sleep restriction as a result of consuming more calories during late-night hours than women. African Americans and Caucasians exhibited similar increases in caloric intake and late-night eating during sleep restriction. Interestingly, there were significant differences between African Americans and Caucasians in macronutrient intake; African Americans consumed more calories from carbohydrates (including more calories from caffeine-free soda and juice) and fewer calories from protein compared to Caucasians during both baseline and sleep restriction. These findings are relevant to the promotion of public health awareness by highlighting nutritional risk factors and modifiable behaviors for weight gain in men and African Americans related to sleep-wake timing.

Our data showing large gender differences in caloric intake are consistent with both population evidence suggesting that men are more susceptible to weight gain resulting from sleep loss (Ko et al., 2007; Meyer et al., 2012; Yang et al., 2013), and with our experimental findings that men gained more weight than women during a sleep restriction protocol (Spaeth et al., 2013). As a result of their larger mass, men eat more than women (Asarian and Geary, 2013); indeed, we found that men consumed more calories during baseline and sleep restriction than women. Importantly, although caloric intake increased during sleep restriction in both genders, men exhibited a larger increase in caloric intake, even after controlling for gender differences in baseline intake. In addition, men displayed a greater delay in meal timing; men consumed a smaller percentage of calories in the morning/afternoon and a larger percentage of calories during late-night hours compared to women during sleep restriction. Meal timing has been identified as a significant contributor to weight maintenance (Allison et al., 2014) and may also underlie the gender differences in the relationship between sleep duration and weight gain/obesity risk.

Sex hormones have been identified as one physiological mechanism underlying gender differences in eating behavior. In women, food intake changes across the menstrual cycle, in part due to changes in estrogen levels (Asarian and Geary, 2013). The effect of sleep loss on the menstrual cycle and estrogen levels in humans is unknown; however, in rats, paradoxical (i.e., REM) sleep deprivation led to a disruption of the estrous cycle and lower estrogen levels (Antunes et al., 2006). We did not systematically assess menstrual cycle phase during the protocol and women were allowed to use oral contraceptives. Given the gender differences we observed, and that estradiol administration has an anorexigenic effect in rodents (Asarian and Geary, 2013), future studies should assess how sleep restriction affects the menstrual cycle and estrogen levels and examine if this relates to changes in food intake. Sleep also influences testosterone levels; plasma levels peak during sleep (a sleep-dependent, not circadian-dependent effect) and experimental sleep deprivation decreases testosterone levels in men (Leproult and Van Cauter, 2011; Carter et al., 2012; Cote et al., 2013). Although decreased testosterone levels have been associated with increased adiposity, there are no studies examining the effect of androgens on food intake in humans (Asarian and Geary, 2013).

The effect of sleep restriction on peripheral physiological controls of eating may also underlie the observed gender differences. Gastric emptying rates and the effect of gastric mechanoreception on food intake varies across the menstrual cycle; in addition, women exhibit slower rates of gastric emptying than men (Asarian and Geary, 2013). Although there is evidence that gastric emptying is delayed during sleep (Pasricha, 2003), the effect of sleep loss on gastric emptying is unknown. Insulin may also play a role; there is mounting evidence that sleep loss is associated with decreased insulin sensitivity (Buxton et al., 2010; Schmid et al., 2011) and intranasal insulin administration decreases food intake in men but not women (Benedict et al., 2008). In addition, St-Onge et al. (2012b) found that experimental sleep restriction with controlled feeding increased the orexigenic hormone ghrelin in men but not women, and noted that this finding was consistent with previous studies showing a significant effect of sleep restriction on ghrelin in men (Spiegel et al., 2004b; Hogenkamp et al., 2013) but not women (Bosy-Westphal et

al., 2008). In rats, ghrelin administration increased food intake to a greater degree in males than females (Asarian and Geary, 2013). Finally, in an experimental study with ad libitum feeding, sleep restriction increased the anorexigenic hormone leptin to a greater degree in women than men (Simpson et al., 2010); moreover, in rats, females are more sensitive to the satiating effects of leptin administration than males (Brown and Clegg, 2010). Future studies with larger sample sizes are needed to confirm these gender differences in peripheral controls of eating and assess how sleep loss affects these signals differently in men and women.

Women may be less vulnerable to environmental stressors than men (Wells, 2000) and sleep deprivation may induce stress-related “comfort food” eating to a greater degree in men than women (Bazhan and Zelena, 2013). However, a recent laboratory study in healthy adults did not find an effect of one week of sleep restriction (6h/night) on 24-h cortisol levels and did not observe gender differences (Pejovic et al., 2013). Thus, sleep restriction in the context of an experiment may not represent an environmental stressor; however, whether stress plays a role in gender differences in the caloric-intake and weight-gain responses to sleep loss outside of the laboratory remains unknown. Gender differences in eating behaviors and attitudes about food are also important to consider. Women scored higher in dietary restraint and in concern over weight control than men (Rolls et al., 1991) and in one study that induced overfeeding, women reduced subsequent caloric intake to a greater extent than men, suggesting they are more sensitive to signals of positive energy balance (Cornier et al., 2004). Chaput et al. (2011a) posited that the association between short sleep duration and weight gain depends on the degree of disinhibited eating in adults. Future research is needed to further elucidate the effect of various eating behaviors, e.g., restraint and disinhibition, on increased caloric intake and weight gain due to sleep restriction and to assess if gender differences in eating behaviors and attitudes relate to differences in the caloric intake and weight response to sleep restriction.

In an earlier study, we found that sleep-restricted African Americans gained more weight than sleep-restricted Caucasians; however, in the current study we did not find differences between African Americans and Caucasians in terms of increased caloric intake or late-night

eating during sleep restriction. In the current study, caloric intake during the first three days of sleep restriction was averaged and used for analyses; therefore, it is possible that African Americans consumed more calories during the final two days following sleep restriction than Caucasians. In addition, the sample size for each race/gender group was smaller in the current study and we may have not been able to detect small differences in intake between African Americans and Caucasians.

Energy expenditure was not assessed in this study, and may explain weight gain differences in the absence of caloric intake differences between African Americans and Caucasians. Physical activity is decreased during the day following sleep restriction (Schmid et al., 2009; Bromley et al., 2012). Although activity levels were relatively low in the current study, it is possible that African Americans were less active than Caucasians, especially since individual differences in total energy expenditure are largely due to variations in non-exercise activity levels (Donahoo et al., 2004). Previous studies have found that African American adults are less active than Caucasian adults under non-sleep deprived and non-laboratory conditions (Vasquez et al., 2013); therefore, future studies should examine if there are race differences in the effect of sleep loss on physical activity levels. Sleep loss can also lead to changes in resting metabolic rate. The extended wakefulness associated with sleep loss leads to an increase in energy expenditure since resting metabolic rate is higher than sleeping metabolic rate (Jung et al., 2011; Shechter et al., 2013; Markwald et al., 2013), and some studies have shown that resting metabolic rate is lower during the day following sleep loss (either after total sleep deprivation or sleep restriction) (Benedict et al., 2011; Buxton et al., 2012; Nedeltcheva et al., 2010). African Americans may exhibit a smaller increase in energy expenditure during extended wakefulness and/or a greater reduction in resting metabolic rate following sleep loss compared to Caucasians. Given that African American adults have a lower sleeping metabolic rate than Caucasian adults under non-sleep deprived conditions (Weyer et al., 1999), future studies should examine if there are race differences in the effect of sleep loss on metabolic rate. Finally, African Americans may exhibit a higher respiratory quotient (lower fat oxidation rate) than Caucasians during sleep restriction;

indeed, respiratory quotient is a contributor to weight gain beyond energy expenditure (Zurlo et al., 1990), and shows race differences, with African Americans having higher respiratory quotients than Caucasians during non-sleep loss conditions (Weyer et al., 1999).

Notably, our subjects consumed a higher percentage of calories from fat and a lower percentage of calories from protein during sleep restriction than baseline, in concordance with previous studies showing an association between sleep loss and increased fat intake (Weiss et al., 2010; St-Onge et al., 2011) and with data from our previous study showing that subjects consumed a greater percentage of calories from fat during late-night caloric intake (Spaeth et al., 2013) (although in that study we found that macronutrient intake did not vary across protocol days). In the current study, subjects consumed more calories from specific food categories including bread, cereal, plain rice and pasta; condiments; desserts; and salty snacks (chips, pretzels and popcorn) during sleep restriction. This finding is consistent with a study reporting that adolescents consumed more foods with a high glycemic index, particularly desserts/sweets, when sleep restricted (Beebe et al., 2013) and a study reporting increased craving for salty and sweet foods in sleep-restricted men (Spiegel et al., 2004b).

We also observed significant race differences in macronutrient intake, with African Americans consuming a lower percentage of calories from protein and a higher percentage of calories from carbohydrates than Caucasians during baseline and sleep restriction. Further examination revealed that African Americans consumed more calories from caffeine-free soda and juice than Caucasians. This finding is consistent with epidemiological studies showing a greater intake of sugar-sweetened beverages in African American children and adults (Bleich et al., 2009; Taveras et al., 2010). Although it is unlikely these differences are exclusively related to sleep restriction, increased intake of carbohydrates, specifically caffeine-free soda and juice, may have contributed to the greater weight gain observed in African Americans in our previous study (Spaeth et al., 2013), as calories from sugar-sweetened beverages contribute to weight gain (Caprio, 2012).

Conclusions

Previous epidemiological and laboratory studies indicate that men and African Americans may be more vulnerable to the weight-gain effect of sleep loss compared to women and Caucasians, respectively. This is the first study to find that men exhibited a greater increase in caloric intake and consumed more calories during late-night hours than women during sleep restriction. Although there were no race differences in daily caloric intake, African Americans consumed more calories from sugar-sweetened beverages than Caucasians, which may relate to greater weight gain in sleep-restricted African Americans. More research is needed, particularly focused on components of energy expenditure and macronutrient utilization rates, to understand other sources of susceptibility to weight gain during sleep loss in African Americans. The current findings extend previous research by identifying increased caloric intake and late-night eating as behavioral mechanisms underlying gender differences in weight gain due to sleep loss. Moreover, our results can be used to promote public health awareness in men and African Americans, by underscoring key risk factors and modifiable behaviors for weight gain related to sleep loss.

ACKNOWLEDGMENTS

This research was supported by NIH grants R01 NR004281 (DFD) and F31 AG044102 (AMS); the Department of the Navy, Office of Naval Research Award No. N00014-11-1-0361 (NG); and Clinical and Translational Research Center (CTRC) grant UL1TR000003. The authors thank the subjects who participated in the experiments and the faculty and staff of the Division of Sleep and Chronobiology who helped acquire the data.

CHAPTER 4: CHRONIC SLEEP RESTRICTION DECREASES RESTING METABOLIC RATE: IMPLICATIONS FOR RACIAL DIFFERENCES IN SLEEP AND OBESITY

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This work has been submitted for publication.

ABSTRACT

Short sleep duration is a significant risk factor for weight gain and obesity, particularly in African Americans and men. Increased caloric intake underlies this relationship but it remains unclear whether decreased energy expenditure is a contributory factor. The objective of the current study was to examine the impact of chronic sleep restriction and recovery sleep on energy expenditure in African American and Caucasian men and women. After two baseline sleep nights, 36 subjects (aged 21 – 50 y) were randomized to an experimental condition (n=25; 4 hours of sleep per night for five nights followed by one night of 12 hours recovery sleep) or a control condition (n=11; 10 hours of sleep per night), in a laboratory protocol with ad libitum food/drink access and limited physical activity. Resting metabolic rate—the largest component of energy expenditure—was decreased after chronic sleep restriction and returned to baseline levels after recovery sleep but did not vary in control subjects. Thus, sleep loss produces energy expenditure changes that contribute to weight gain in healthy adults. Relative to Caucasians (n=9), African Americans (n=16) exhibited a lower resting metabolic rate after baseline sleep, sleep restriction and recovery sleep, a smaller rebound in resting metabolic rate after recovery sleep, and a higher respiratory quotient (indicating decreased lipid metabolism) after sleep restriction – these changes collectively place them at heightened risk for weight gain and obesity. African Americans, who consistently report a higher prevalence of habitual short sleep duration, may need to compensate for these energy expenditure deficits by reducing caloric intake or increasing physical activity to prevent weight gain.

INTRODUCTION

Epidemiological and laboratory studies have consistently found that short sleep duration is a significant risk factor for weight gain and obesity. Cross-sectional and prospective cohort studies indicate that short sleep duration is associated with a higher body mass index (BMI) (Ford et al., 2014; Moraes et al., 2013), an increased risk for obesity (Singh et al., 2005; Di Milia et al., 2013), and is a reliable predictor of greater weight gain (Hasler et al., 2004; Chaput et al., 2008; Kobayashi et al., 2012; Markwald et al., 2013; Spaeth et al., 2013). The association between short sleep duration and increased odds for obesity is stronger in African Americans and men than in Caucasians and women, respectively (Ko et al., 2007; Meyer et al., 2012; Yang et al., 2013; Donat et al., 2013; Grandner et al., 2014). Increased caloric intake underlies the relationship between short sleep duration and weight gain (Bosy-Westphal et al., 2008; Brondel et al., 2010; St-Onge et al., 2011; Calvin et al., 2013; Markwald et al., 2013; Spaeth et al., 2013), but it remains unclear whether decreased energy expenditure —an important contributor to energy balance and weight management (Filozof and Gonzalez, 2000; Piaggi et al., 2013) — also plays a role.

Total energy expenditure is the sum of three components: resting metabolic rate (RMR; energy needed for cell repair, immune activity, thermoregulation, etc.), diet-induced thermogenesis (DIT; heat production caused by the intake and digestion of food) and physical activity levels (muscular work required for all spontaneous and willful movements) (Leonard, 2012). Sleep loss can produce a change in energy expenditure by affecting each or all of these components. Because energy expenditure during sleep is lower than energy expenditure during wake (Penev, 2012), the increased energy requirement associated with sleep restriction will lead to negative energy balance and weight loss over time (if diet is held constant). To protect against this state, it is hypothesized that the body employs neuroendocrine, metabolic and behavioral compensatory responses to increase energy intake and conserve energy (Penev, 2012). If more energy is consumed and conserved than necessary (i.e. over-compensation), a state of positive energy balance and weight gain will occur over time.

Several studies support this compensatory hypothesis. The additional energy required for extended wakefulness from acute total sleep deprivation and sleep restriction is 135 kcal/day (Jung et al., 2011) and ~100 kcal/day (Markwald et al., 2013; Shechter et al., 2013), respectively. Under ad libitum feeding conditions, subjects consume ~500 additional calories when sleep restricted (Spaeth et al., 2013); thus, during extended wakefulness healthy adults overcompensate for increases in energy cost with marked increases in energy intake. In addition, some laboratory studies, but not all (Bosy-Westphal et al., 2008; Brondel et al., 2010; Hursel et al., 2011; Nedeltcheva et al., 2009b; St-Onge et al., 2011; Shechter et al., 2014), show decreased energy expenditure during the day following sleep loss, including reductions in RMR (Nedeltcheva et al., 2010; Benedict et al., 2011; Buxton et al., 2012), DIT (Benedict et al., 2011), and physical activity (Schmid et al., 2009; Bromley et al., 2012), suggesting a metabolic adaptation to conserve energy in response to the previous day's extended wakefulness. Substrate utilization, the type of fuel used for metabolism, also contributes to energy expenditure and is assessed using the respiratory quotient (RQ; CO2 expired / O2 consumed) (McClave et al., 2003). RQ ranges from 0.63 (0.7 associated with fat metabolism) to 1.3 (1.0 associated with carbohydrate metabolism) and higher RQ values are associated with greater weight gain (Marra et al., 2004; Piaggi et al., 2013). Some studies have found that sleep loss increases RQ (Nedeltcheva et al., 2010; Hursel et al., 2011), while others have found it has no effect on RQ (Jung et al., 2011; Shechter et al., 2014).

Only three studies have assessed the impact of recovery sleep on components of energy expenditure, with inconsistent results. Sleeping metabolic rate was lower during recovery sleep following acute total sleep deprivation than baseline sleep in a small sample of young healthy men and women; however, RQ did not change (Jung et al., 2011). Total energy expenditure and RMR did not change when assessed during days following baseline sleep, sleep restriction and recovery sleep in a small sample of young healthy women (Bosy-Westphal et al., 2008). Finally, RMR decreased following 3 weeks of sleep restriction combined with circadian disruption and then returned to baseline levels after 9 nights of recovery sleep in a sample of young healthy men

and women (Buxton et al., 2012). Many of the previous studies examining the effect of sleep loss and recovery sleep on energy expenditure controlled for caloric intake (limiting subject's to a standard amount of calories per day) which does not mimic what occurs when adults are sleep restricted outside of the laboratory; therefore, an experiment examining the energy expenditure effects of chronic sleep restriction under ad libitum feeding conditions is needed.

Importantly, African Americans and men may be more vulnerable to increased weight gain resulting from sleep loss than Caucasians and women, respectively (Ko et al., 2007; Meyer et al., 2012; Yang et al., 2013; Donat et al., 2013; Grandner et al., 2014). No studies have examined race or gender differences in the energy expenditure response to sleep restriction. We found that men increased caloric intake to a greater degree than women during sleep restriction whereas African Americans and Caucasians exhibited comparable caloric intake levels (Spaeth et al., 2014a). Given that African Americans gain more weight than Caucasians during sleep restriction (Spaeth et al., 2013) but do not show greater caloric intake, it is likely that chronic sleep restriction affects energy expenditure differently in these racial groups.

We investigated the impact of chronic sleep restriction and subsequent recovery sleep on energy expenditure measures in a sample of healthy African American and Caucasian men and women. We hypothesized that resting metabolic rate and diet-induced thermogenesis would be reduced and respiratory quotient would be increased following chronic sleep restriction and that these measures would show a compensatory return to baseline levels after a night of recovery sleep, indicating energy expenditure contributes to the relationship between short sleep duration and obesity in healthy adults. Considering that African Americans gain more weight but exhibit a similar increase in caloric intake compared to Caucasians when sleep restricted, we hypothesized that resting metabolic rate would be lower and that respiratory quotient would be higher following sleep restriction and recovery sleep in African Americans than in Caucasians.

MATERIALS AND METHODS

Subjects

Healthy individuals, aged 21–50 y, were recruited in response to study advertisements. They reported habitual nightly sleep durations between 6.5h and 8.5h, habitual bedtimes between 2200h and 0000h, and habitual morning awakenings between 0600h and 0900h; these reports were confirmed objectively using actigraphy. They had no evidence of habitual napping, no sleep disturbances (i.e., no complaints of insomnia, daytime sleepiness, or other sleep-wake disturbances), and an absence of extreme morningness or extreme eveningness, as assessed by questionnaire (Smith et al., 1989). Subjects were free of acute and chronic medical and psychological conditions, as established by interviews, clinical history, questionnaires, physical examinations, and blood (including a fasting blood glucose test) and urine tests. They were nonsmokers and did not participate in shift work, transmeridian travel, or irregular sleep-wake routines in the 60 days prior to the study. Enrolled subjects were monitored at home with actigraphy, sleep-wake diaries, and time-stamped call-ins to assess bedtime and waketime during the week prior to the in-laboratory phase and the week after the laboratory phase. They were not permitted to use caffeine, alcohol, tobacco, and medications (except oral contraceptives) in the week before the laboratory study, as verified by urine screenings. Sleep disorders were excluded by a night of laboratory polysomnography and oximetry measurements. The studies were approved by the Institutional Review Board of the University of Pennsylvania. All subjects provided written informed consent before enrollment and were compensated for their participation.

Experimental Design

Subjects participated in one of two protocols in the Sleep and Chronobiology Laboratory at the Hospital of the University of Pennsylvania and were studied for 14 or 18 consecutive days continuously, in a laboratory protocol with limited physical activity, with daily clinical checks of vital signs and symptoms by nurses (with an independent physician on call). Subjects were randomized to either the sleep restriction or control condition. In both protocols, the sleep

restriction condition consisted of two initial baseline nights of 10h or 12h TIB per night (BL1-2; 2200h–0800h/1000h) followed by five nights of sleep restricted to 4h TIB per night (SR1-5; 0400h–0800h) and one night of 12h TIB recovery sleep (R1; 2200h-1000h; **Figure 4.1**). We selected this dose of chronic sleep restriction because it produces cumulative neurobehavioral deficits in most healthy adults (Van Dongen et al., 2013) and is within the range of sleep loss that occurs as a result of lifestyle factors (Luckhaupt et al., 2010; Knutson et al., 2010). The control condition consisted of 10h TIB per night (2200h-0800h) each night (**Figure 4.1**).

Subjects were not permitted to leave the laboratory. During the study, subjects were ambulatory and were permitted to watch television, read, play video or board games, and perform other sedentary activities between test bouts (which were completed while sitting at a computer) but they were not allowed to exercise. Subjects wore a wrist actigraph throughout the in-laboratory protocol. On certain protocol days, subjects wore ambulatory electroencephalography (EEG) and electrocardiography (ECG) recording equipment for 24-h intervals. The light levels in the laboratory were held constant at < 50 lux during scheduled wakefulness and < 1 lux during scheduled sleep periods. Ambient temperature was maintained between 22°- 24°C. Subjects were behaviorally monitored by trained staff continuously throughout the protocol to ensure adherence. Food/drink was ad libitum throughout the protocol.

Body composition and metabolic measurements were collected after an overnight fast in the morning following the first night of baseline sleep, after the fifth night of either sleep restriction or the corresponding night in the control condition and after the night of recovery sleep or the corresponding night in the control condition (**Figure 4.1**). To control for time-of-day effects, measurements took place during the two hours following waketime (between 0800h-1000h on BL1, SR5/CD5 and CD6 and between 1000h-1200h on R1). Prior to the measurement following the fifth night of sleep restriction, subjects were awake for 6h of the 10h fast (2200h-0359h) and were only allowed to consume water during this time. Upon awakening, subjects were instructed to use the restroom. Each subject's body composition was assessed and then subjects remained in bed in a supine position for the remainder of the testing period. After the body composition

measurement, each subject's metabolic rate was assessed twice, first after the overnight fast (RMR) and again 30 minutes after consuming a standardized meal (in order to calculate DIT; **Figure 4.1**). The RQ measurement from the first test (fasted) was used for analyses.

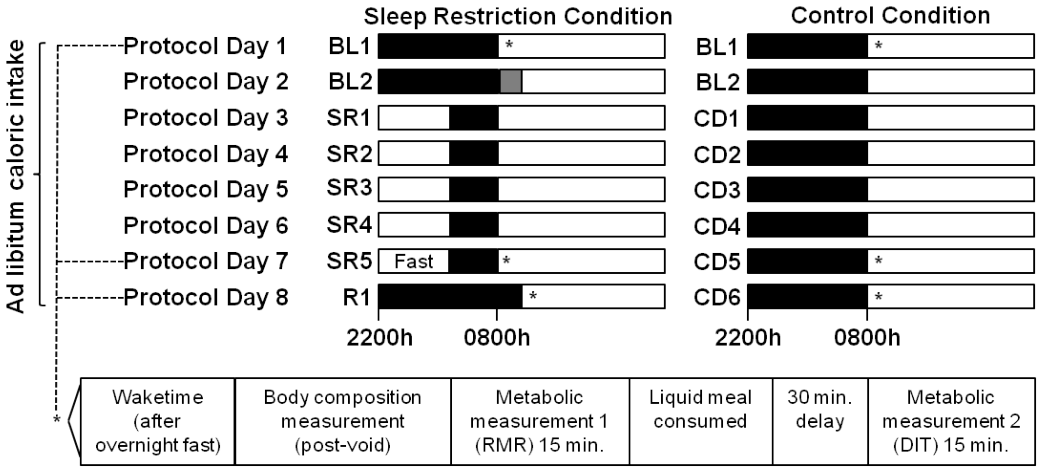

FIGURE 4.1: Protocol Schedule

The sleep restriction condition consisted of two initial baseline nights of sleep followed by five nights of sleep restricted to 4h time-in-bed (TIB) per night and one night of recovery sleep. The control condition consisted of 8 consecutive nights of 10h TIB per night. Food/drink intake was ad libitum; however, prior to the measurement on SR5, sleep-restricted subjects were awake for 6h of the 10h overnight fast (2200h-0359h) and were only allowed to consume water during this time. *Body composition and metabolic measurements were collected during the morning after the first night of baseline sleep, after the fifth night of sleep restriction and after the night of recovery sleep, or after corresponding nights in the control condition. To control for time-of-day effects, measurements took place during the two hours following waketime (between 0800h-1000h on BL1, SR5/CD5 and CD6 and between 1000h-1200h on R1). Upon awakening, subjects used the restroom. Each subject's body composition was assessed and then subjects remained in bed in a supine position for the remainder of the testing period. After the body composition measurement, each subject's metabolic rate was assessed twice, first after the overnight fast (resting metabolic rate measurement [RMR] and respiratory quotient [RQ]) and again 30 minutes after consuming a standardized meal (in order to calculate diet-induced thermogenesis [DIT]).

Measures

Each subject's body composition was measured using bioelectrical impendence analysis (Omron HBF-510W, 4-Limb device), following voiding. Body weight, fat percentage, and fat free mass (FFM) were collected.

Metabolic rate (kcal/day) was measured using indirect calorimetry with a ventilated hood system (TrueOne 2400 metabolic cart, Parvo Medics, Sandy, UT, USA). Before each measurement day, a flow meter calibration was conducted and before each use, the metabolic cart was calibrated with reference gas. After achieving steady state (approximately 5 minutes), expired gases were collected for 10 minutes and used to calculate metabolic rate. Trained technicians monitored subjects during the test and instructed them to keep their eyes open in order to ensure that they remained awake during the tests. After completing the first metabolic rate assessment (RMR and RQ), subjects consumed a standardized liquid meal: 5.5g/100 ml protein (15% of total kcal), 21.1 g/100 ml carbohydrate (57% of total kcal), 4.6 g/100 ml fat (28% of total kcal) (Ensure Plus, Abbott Laboratories, Abbott Park, IL, USA), based on each subject's fat free mass (30% of 30 kcal/kg FFM). Metabolic rate was assessed again 30 minutes after each subject consumed the liquid meal. DIT was calculated as the difference in the two metabolic rate measurements divided by the amount of calories consumed in the liquid meal (%; Measurement 2 – Measurement 1 / calories consumed in liquid meal) (Westerterp, 2004).

Statistical Analyses

Repeated measures analysis of variance (ANOVAs) compared changes across protocol days for RMR, RQ and DIT; planned comparisons were then conducted using paired-sample t-tests and Wilcoxon signed rank tests. Multivariate ANOVAs with race and gender as independent variables and age and FFM as covariates compared group differences in RMR, RQ and DIT during each measurement. Effect sizes were calculated using Cohen's d; when calculating effect size for changes within-subjects, a correction for dependence between means was used (Cohen, 1988; Morris and DeShon, 2002).

RESULTS

Subject Characteristics

Sleep-restricted subjects (n=25) did not differ from control subjects (n=11) in age (P = 0.47), BMI (P = 0.45), fat-free mass (P = 0.85), or in the percentage of African Americans (P = 0.60) or females (P = 0.68) (**Table 4.1**). Sleep duration and timing during the week prior to the in-laboratory study were assessed using wrist actigraphy; there were no differences in pre-study sleep duration, onset, offset or midpoint between sleep-restricted and control subjects (all P > 0.10). Sleep-restricted subjects gained 1.12 ± (SD) 1.34 kg from BL1 to SR5, a gain that was significantly different from 0 (t (24) = 4.15, P < 0.001). The change in weight in control subjects (0.62 ± 1.33) from BL1 to CD5 did not significantly differ from 0 (P = 0.15).

Sleep-restricted African Americans and Caucasians did not differ in age (P = 0.93), BMI (P = 0.96), fat-free mass (P = 0.97) or the percentage of women (P = 0.98). Sleep-restricted men and women did not differ in age (P = 0.13), BMI (P = 0.68), or in the percentage of African Americans (P = 0.98); however, men had greater fat-free mass than women (men: 62.91 ± 8.25 kg, women: 41.18 ± 3.07 kg; t (23) = 8.27, P < 0.001). There were no gender or race differences in pre-study sleep duration, onset, offset or midpoint (all P > 0.38).

TABLE 4.1. Subject characteristics (Mean ± SD)

	Age (y)	BMI	Fat-Free Mass (kg)	% African Americans (n)	% Women (n)
Sleep-Restricted Subjects (n=25)	31.76 ± 8.02	25.20 ± 3.01	53.35 ± 12.73	64.0% (16)	44.0% (11)
Control Subjects (n=11)	34.00 ± 9.37	24.34 ± 3.41	52.52 ± 9.65	54.5% (6)	36.4% (4)

There were no significant differences between condition groups (all P > 0.05)

Resting Metabolic Rate

RMR varied across the three measurements in sleep-restricted subjects (F (2, 48) = 3.96, P = 0.026) but not in control subjects (P = 0.42). In sleep-restricted subjects, RMR was lower on

SR5 compared to BL1 (t (24) = 2.22, *P* = 0.036, d = 0.50, decreased by 2.3%), and higher on R1 compared to SR5 (t (24) = 2.82, *P* = 0.01, d = 0.56, increased by 2.8%) but did not differ between BL1 and R1 (*P* = 0.89); these comparisons were also significant when using non-parametric tests (BL1 to SR5: *P* = 0.045, SR5 to R1: *P* = 0.014) (**Figure 4.2A**).

In sleep-restricted subjects, using a multivariate ANOVA with gender and race as independent variables and age and fat-free mass as covariates, African Americans exhibited a lower RMR during each measurement than Caucasians (BL1: F (1, 19) = 6.96, *P* = 0.016, d = 0.47; SR5: F (1, 19) = 4.84, *P* = 0.04, d = 0.38; R1: F (1, 19) = 8.62, *P* = 0.008, d = 0.62, **Figure 4.3A**) and although African Americans did not differ from Caucasians in the change in RMR from BL1 to SR5 (*P* = 0.29), they exhibited a smaller increase in RMR from SR5 to R1 (F (1, 19) = 4.59, *P* = 0.045, d = 0.96). By contrast, RMR did not differ between men and women during each measurement (BL1: *P* = 0.06, SR5: *P* = 0.23, R1: *P* = 0.09) or in the change in RMR from BL1 to SR5 (*P* = 0.22) or from SR5 to R1 (*P* = 0.17).

Respiratory Quotient

RQ varied across the three measurements in sleep-restricted subjects (F (2, 48) = 8.22, *P* = 0.001) and in control subjects (F (2, 48) = 6.73, *P* = 0.006). For both groups, RQ was higher on SR5/CD5 (sleep-restricted: t (24) = 3.39, *P* = 0.002; control: t (10) = 2.81, *P* = 0.019) and R1/CD6 (sleep-restricted: t (24) = 2.96, *P* = 0.007; control: t (10) = 3.05, *P* = 0.012) compared to BL1, but did not differ between SR5/CD5 and R1/CD6 (sleep-restricted: *P* = 0.82; control: *P* = 0.22), the same pattern was observed when using non parametric tests (BL1 to SR5/CD5: all *P* < 0.05, BL1 to R1/CD6: all *P* < 0.05, SR5/CD5 to R1/CD6 all *P* > 0.20) (**Figure 4.2B**).

In sleep-restricted subjects, using a multivariate ANOVA with gender and race as independent variables and age and fat-free mass as covariates, African Americans exhibited a higher RQ than Caucasians during SR5 (F (1, 19) = 4.93, *P* = 0.039, d = 0.70, **Figure 4.3B**), but did not differ from Caucasians in RQ during BL1 (*P* = 0.35) or R1 (*P* = 0.83). There were no differences between men and women in RQ during each measurement (BL1: *P* = 0.87, SR5: *P* = 0.21, R1: *P* = 0.30).

Diet-Induced Thermogenesis

DIT did not vary across the three measurements in sleep-restricted subjects (P = 0.065) or in control subjects (P = 0.61) (**Figure 4.2C**). In sleep-restricted subjects, the multivariate ANOVA with gender and race as independent variables and age and fat-free mass as covariates revealed no race or gender differences in DIT for each measurement (all P > 0.10).

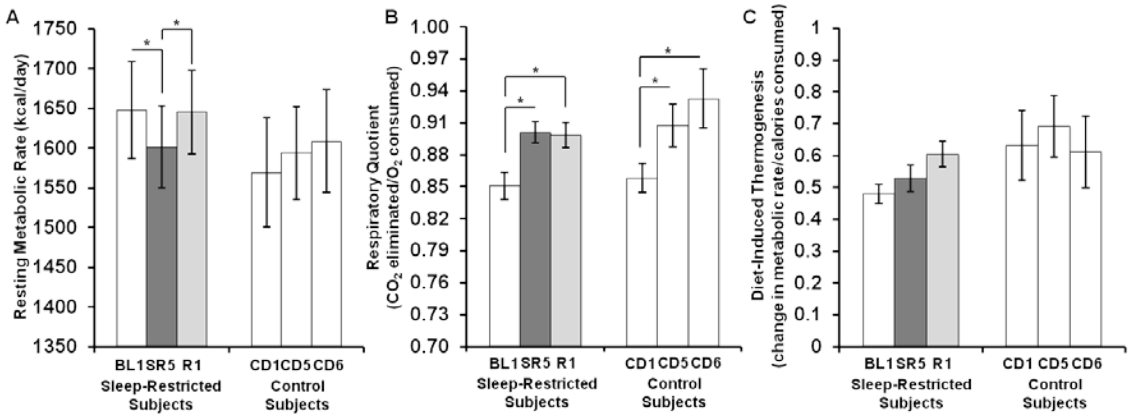

FIGURE 4.2: Energy Expenditure Measures across Protocol Days

(**A**) Resting metabolic rate varied across the three measurements in sleep-restricted subjects (P = 0.026) but not in control subjects. In sleep-restricted subjects, resting metabolic rate was lower on SR5 compared to BL1 (P = 0.036, d = 0.50, decreased by 2.3%), and higher on R1 compared to SR5 (P = 0.01, d = 0.56, increased by 2.8%) but did not differ between BL1 and R1. (**B**) Respiratory quotient varied across the three measurements in sleep-restricted subjects (P = 0.001) and in control subjects (P = 0.006). For both groups, respiratory quotient was significantly higher on SR5/CD5 (sleep-restricted: P = 0.002; control: P = 0.019) and R1/CD6 (sleep-restricted: P = 0.007; control: P = 0.012) compared to BL1, but did not differ between SR5/CD5 and R1/CD6. (**C**) Diet-induced thermogenesis did not vary across the three measurements in sleep-restricted subjects or control subjects. Data expressed as mean ± SEM, *P < 0.05.

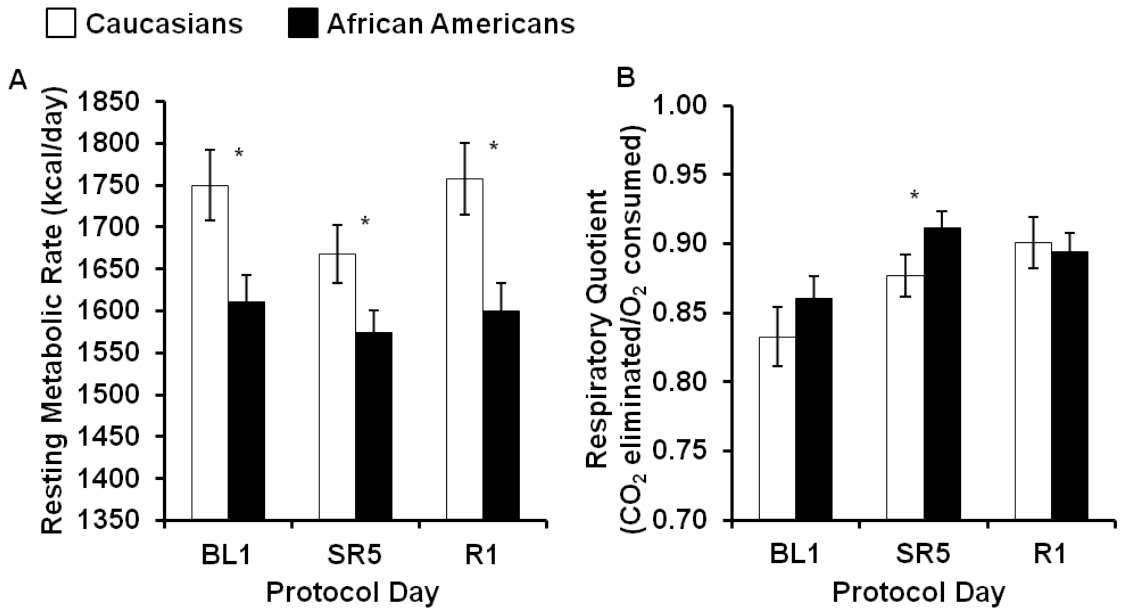

FIGURE 4.3: Race Differences in Resting Metabolic Rate and Respiratory Quotient

(**A**) Sleep-restricted African Americans exhibited a lower resting metabolic rate during each measurement (BL1: $P = 0.016$, d = 0.47; SR5: $P = 0.04$, d = 0.38; R1: $P = 0.008$, d = 0.62) than Caucasians when covarying age and fat-free mass. African Americans did not differ from Caucasians in the change in resting metabolic rate from BL1 to SR5; however, African Americans exhibited a smaller increase in resting metabolic rate from SR5 to R1 ($P = 0.045$, d = 0.96). (**B**) Respiratory quotient was higher in African Americans than Caucasians after sleep restriction ($P = 0.039$, d = 0.70) but did not differ between groups after baseline sleep or recovery sleep. Data expressed as mean ± SEM, $^*P < 0.05$.

DISCUSSION

Sleep loss produced energy expenditure changes that contribute to weight gain in healthy adults (Filozof and Gonzalez, 2000; Piaggo et al., 2013), with greater impairments in African Americans, placing this group at heightened risk for obesity. Resting metabolic rate (RMR)—the largest (60-70%) component of energy expenditure—was significantly reduced after chronic sleep restriction and rebounded to baseline levels after one night of recovery sleep. Respiratory

quotient (RQ), a marker of fuel metabolism, increased after chronic sleep restriction and remained elevated, indicating a sustained reduction in lipid metabolism rate. Importantly, African Americans exhibited a lower RMR after baseline sleep, sleep restriction and recovery sleep, a smaller rebound in RMR after recovery sleep, and a higher RQ after sleep restriction compared to Caucasians. African Americans—who consistently report shorter sleep durations in population studies—may need to compensate for these energy expenditure deficits from sleep loss by reducing caloric intake or increasing physical activity in order to prevent obesity.

We found that RMR decreased after chronic sleep restriction and returned to baseline levels after recovery sleep. This is consistent with a study conducted by Buxton et al. (2012) showing reductions in RMR following three weeks of sleep restriction (5.6 hours of sleep per 24h) and forced desynchrony (28h “days”), and an increase in RMR to baseline levels following 9 nights of recovery sleep with circadian re-entrainment (10 hours of sleep per 24h) in young healthy adults under controlled caloric intake conditions. However, that study’s design did not reflect the food availability or type of sleep loss typically experienced by adults outside of the laboratory and made it impossible to determine whether sleep restriction, circadian disruption, or their combination, led to the observed changes in RMR. Our results demonstrate that sleep restriction, without significant circadian disruption and under ad libitum feeding conditions, plays an important role in RMR regulation. Furthermore, the Buxton study (2012) could not determine how rapidly RMR rebounds following recovery sleep, we found that RMR returned to baseline levels after only one night of recovery sleep which is consistent with the time-course of the caloric intake response (Spaeth et al., 2013). Thus, ensuring adequate recovery sleep, even for only one night, following sleep loss is a critical intervention for mitigating weight gain and obesity risk.

Although our results related to resting metabolic rate changes are consistent with previous findings (Nedeltcheva et al., 2010; Benedict et al., 2011; Buxton et al., 2012), other studies have found no effect of sleep restriction on resting metabolic rate (Bosy-Westphal et al., 2008; Nedeltcheva et al., 2009; St-Onge et al., 2011; Shechter et al., 2013) when assessed the day following sleep loss. Although it is unclear why there are discrepant findings related to the

effects of sleep loss on measures of energy expenditure, differences in study design likely play a role. For example, allowing subjects to: leave the laboratory to walk outside (Nedeltcheva et al., 2009), consume caffeine daily during the study (Nedeltcheva et al., 2009; St-Onge et al., 2011), use a gym at their leisure (St-Onge et al., 2011) and sleep at home rather than supervised in the laboratory (Bosy-Westphal et al., 2008) may have confounded the assessment of resting metabolic rate in previous studies. Although more research is needed in this area, decreased energy expenditure during the day following sleep loss (reductions in resting metabolic rate and physical activity) may play a role in the relationship between sleep restriction and weight gain/obesity.

One theory posits that sleep restriction produces weight gain due to the overcompensation of neuroendocrine, metabolic and behavioral responses aimed to increase energy intake and conserve energy in order to counteract the additional energy requirement associated with extended wakefulness (Penev, 2012). Our study provides further support for this hypothesis. Under non-sleep restriction conditions, RMR increases with positive energy balance: short-term (2-7 days) overfeeding leads to weight gain (+0.5 – 1.8 kg) and increased RMR (+1.1 – 11.7%) (Harris et al., 2006). We have now established that, under ad libitum feeding conditions, chronic sleep restriction leads to increased caloric intake (+500 kcal) during extended wakefulness (Spaeth et al., 2013) and weight gain (+1.12 kg), but *decreased* RMR (-2.3%) the following day. Thus, sleep restriction produces metabolic responses to conserve energy – this decrease in energy expenditure likely contributes to the weight gain effect of sleep loss.

Consistent with previous studies (Nedeltcheva et al., 2010; Hursel et al., 2011), sleep restriction increased RQ, which has been associated with weight gain (Marra et al., 2004; Piaggi et al., 2013). This increase, however, was also observed in control subjects, suggesting that factors other than sleep restriction, such as living in a sedentary laboratory environment, contributed to this effect. DIT did not change after sleep restriction or recovery sleep, in agreement with a previous study which found no effect of sleep restriction on DIT in healthy women (Shechter et al., 2014). However, due to the time constraints of our protocol, DIT was

based on only one measurement (30 minutes following ingestion of a standardized liquid meal). Moreover, given that acute total sleep deprivation decreases DIT (Benedict et al., 2011); future studies are needed to clarify the effects of sleep restriction on DIT.

Epidemiological studies show a stronger association between short sleep duration and increased odds for obesity in African Americans than in Caucasians (Donat et al., 2013; Grandner et al., 2014) and African Americans gained more weight than Caucasians in a controlled sleep restriction experiment (Spaeth et al., 2013). We have also observed that African Americans exhibit similar increases in caloric intake compared to Caucasians (Spaeth et al., 2014a). In the current study, we found that African Americans exhibited a lower RMR than Caucasians following baseline sleep, five nights of sleep restriction and one night recovery sleep. This suggests that African Americans gain more weight than Caucasians when sleep restricted because they exhibit similar levels of energy intake but lower levels of energy expenditure. African Americans also do not benefit metabolically from recovery sleep to the same degree as Caucasians; Caucasians exhibited a larger increase in RMR after recovery sleep than African Americans. Finally, African Americans exhibited a higher RQ after sleep restriction which reflects increased carbohydrate metabolism (decreased lipid metabolism) and is associated with weight gain (Marra et al., 2004; Piaggi et al., 2013). Under non-sleep restriction conditions, African Americans exhibit a lower RMR and sleeping metabolic rate (Weyer et al., 1999; Gannon et al., 2000) and less physical activity (Vasquez et al., 2007) than Caucasians. Collectively, these findings suggest that if caloric intake is comparable between races, African Americans are at greater risk for weight gain and obesity due to reduced energy expenditure, which may become exacerbated with sleep loss.

Limitations

Our study has a few limitations. We used a metabolic cart with a 15-minute test duration to assess metabolic rate and only one time point (30 minutes following consumption of the standardized meal) to estimate DIT. Whole-room indirect calorimetry systems with continuous recordings for multiple hours provide a more complete measure of postprandial elevations in energy expenditure (Shechter et al., 2014). In addition, physical activity was not directly assessed

in the current study, since it was held at a low level within the confines of our laboratory; this measure may also show race or gender differences. Finally, in our female subjects we did not systematically monitor the menstrual cycle which can influence energy balance; in addition, women were allowed to use oral contraceptives during the study.

Conclusions

Chronic sleep restriction decreased resting metabolic rate in healthy adults, suggesting that energy expenditure does play a role in the relationship between short sleep duration and weight gain. In population studies, African Americans report a higher prevalence of habitual short sleep duration (Singh et al., 2005; Hale et al., 2007; Whinnery et al., 2014), and our results show for the first time, that this racial group exhibits a lower resting metabolic rate before, during, and after sleep restriction than Caucasians, placing them at heightened risk for weight gain and obesity.

ACKNOWLEDGMENTS

This research was supported by NIH grants R01 NR004281 (DFD) and F31 AG044102 (AMS); the Department of the Navy, Office of Naval Research Award No. N00014-11-1-0361 (NG); and Clinical and Translational Research Center (CTRC) grant UL1TR000003. The authors thank the subjects who participated in the experiments and the faculty and staff of the Division of Sleep and Chronobiology who helped acquire the data.

CHAPTER 5: EFFECT OF SHORT-TERM FASTING ON LATE-NIGHT NEUROBEHAVIORAL PERFORMANCE DURING SLEEP RESTRICTION

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ABSTRACT

Evidence suggests that caloric intake leads to decreased alertness and increased sleepiness. Healthy adults consume approximately 500 additional calories during late-night hours (2200h-0359h) when sleep restricted. Late-night/early-morning hours are also when most sleep-restricted adults display pronounced decreases in alertness and neurobehavioral performance deficits. The objective of the current study was to assess if refraining from consuming late-night calories would affect neurobehavioral performance during sleep restriction. Forty-four subjects (aged 21 – 50 y) had ad libitum access to food/drink during the first three days following sleep restriction (4 hour sleep opportunity per night, 0400h-0800h). Beginning at 2200h on the fourth day following sleep restriction, subjects assigned to the fed condition (n=20) continued to have ad libitum access to food/drink whereas subjects assigned to the fasted condition (n=24) were only allowed to consume water. All subjects completed assessments of vigilant attention, working memory and cognitive throughput as well as subjective measures of sleepiness, fatigue, stress and mood at 0200h. On the fourth day following sleep restriction, subjects in the fed condition exhibited poorer performance on the Psychomotor Vigilance Test (i.e., slower reaction times and more errors of omission) than subjects in the fasted condition; however, subjects in the fed and fasted conditions did not differ on tasks of working memory and cognitive throughput or on subjective measures of sleepiness, fatigue, stress or mood. These results indicate that refraining from consuming calories during late-night hours may be a useful strategy for alleviating (but not eliminating) decreased vigilance during sleep restriction.

INTRODUCTION

The prevalence of short sleepers (≤6 hours/day) among U.S. adults has increased significantly in recent decades (Knutson et al., 2010; Luckhaupt et al., 2010). Most habitual short sleepers require the same amount of sleep as adults who habitually sleep 7-8 h/night; thus, short sleepers gradually accrue sleep debt over time (Aeschbach et al., 1996; Klerman and Dijk, 2005; Bradshaw et al., 2007). This type of chronic partial sleep deprivation leads to neurobiological and physiological changes that can impede performance and negatively impact health.

Human performance is optimal when homeostatic drives for sleep are low and the circadian propensity for wakefulness is high (Van Dongen and Dinges, 2005; Raslear et al., 2011). The homeostatic sleep-dependent process (process S) balances sleep propensity by keeping track of recent sleep history (Daan et al., 1984); sleep propensity is increased as hours of wakefulness increase across the day. A second homeostatic process (process U) influences waking performance by monitoring sleep-wake on a longer time scale (nightly sleep duration); process U builds up over several days of prolonged wakefulness and dissipates during sleep (McCauley et al., 2009; McCauley et al., 2013). The endogenous circadian process (process C) tracks changes in light exposure (as well as other zeitgebers) and entrains sleep propensity to the light-dark cycle; sleep propensity is increased during the night in humans (Czeisler et al., 1999). These processes interact to form a dynamic relationship between sleep propensity and waking performance. Performance is particularly degraded during late-night / early-morning hours (0200h-0800h) (Goel et al., 2013) and as sleep debt accumulates across each night of sleep restriction, impairment in sustained attention, working memory and cognitive throughput becomes more severe (Belenky et al., 2003; Van Dongen et al., 2003).

Chronic sleep restriction, due to habitually sleeping less than 7 hours/night, also negatively impacts health. Short sleep duration has been associated with increased risk for obesity (Di Milia et al., 2013; Ford et al., 2014) and diseases associated with obesity such as type 2 diabetes (McNeil et al., 2013; Merikanto et al., 2013) and hypertension (Faraut et al., 2012; Zou et al., 2013). Laboratory studies have found that sleep restriction leads to weight gain and an

increase in daily caloric intake that exceeds the amount of calories needed for energy balance (Markwald et al., 2013; Spaeth et al., 2013). The increase in daily caloric intake is primarily due to the consumption of additional food/drink during late-night hours (2200h-0359h) and the percentage of calories derived from fat is significantly high in late-night calories compared to calories consumed during morning, afternoon and evening hours (Spaeth et al., 2013). Interestingly, this late-night time period coincides with the marked decline in neurobehavioral performance that occurs during sleep restriction (Goel et al., 2013). Previous studies from our laboratory (Dinges et al., 1997; Van Dongen et al., 2003; Goel et al., 2009a; Banks et al., 2010) and others (Belenkey et al., 2003; Bliese et al., 2006; Cote et al., 2008, Fafrowicz et al., 2010; Rupp et al., 2009; Wu et al., 2010; Pejovic et al., 2013) assessed neurobehavioral performance during sleep restriction under ad libitum food/drink conditions. Whether or not the consumption of additional calories during late-night hours contributes to decreased neurobehavioral performance has not been tested.

The postprandial period has been associated with a restful state and sleepiness (postprandial somnolence) whereas hunger has been associated with activation and arousal (Leigh Gibson and Green, 2002). Rodents display increased wakefulness and locomotor activity when food deprived (Borbely, 1977; Danguir and Nicolaidis, 1979, Deswasmes et al., 1989; Itoh et al., 1990; Challet et al., 1997) whereas ingestion of a cafeteria diet (high in calories, particularly those derived from fat and carbohydrates) increases sleep (Danguir, 1987; Hansen et al., 1998). In humans, ingestion of food, particularly fat, is associated with increased subjective sleepiness, decreased sleep latency and increased sleep duration as well as decreased subjective alertness and poorer sustained attention performance (Craig and Richardson, 1989; Harnish et al., 1998; Reyner et al., 2012; Wells et al., 1995; Wells and Read, 1996; Wells et al., 1997; Wells et al., 1998; Zammit et al., 1995). The ingestion of the mid-day meal contributes to the decrease in alertness that occurs early in the afternoon, known as the “post-lunch dip” (Smith and Miles, 1986a,b; Bes et al., 2009).

Results from previous studies examining the short-term effect of fasting on neurobehavioral performance in adults are inconsistent, with studies reporting improvements, no change, or decrements on tasks of reaction time, working memory, attention and executive function (Benau et al., 2014). Discrepancies are likely due to what aspect of performance is being assessed, the timing and duration of the fast, and the subject's age and nutritional status (Kanarek, 1997; Leigh Gibson and Green, 2002; Gomez-Pinilla, 2008; Benau et al., 2014). Time of day seems to be particularly important; fasting may be more beneficial for improving performance in the afternoon than in the morning (Kanarek, 1997; Benau et al., 2014). Although there is a paucity of data available on the effects of late-night fasting versus eating, one study found that nighttime intake reduced the detection of targets in a cognitive vigilance task (Smith and Miles, 1986c).

The aforementioned lines of evidence suggest that late-night eating/drinking may induce sleepiness and add to the neurobehavioral deficits caused by sleep restriction; however, few controlled experiments have manipulated food availability during late-night hours and assessed neurobehavioral performance. To address this deficiency, we experimentally tested the hypothesis that the neurobehavioral deficits caused by sleep restriction would be greater in subjects consuming food/drink ad libitum during late-night hours than in subjects only allowed to consume water during this time. Subjective ratings of sleepiness, stress and mood were also measured.

MATERIALS AND METHODS

Subjects

Healthy individuals, aged 21–50 y, were recruited in response to study advertisements. They reported habitual nightly sleep durations between 6.5h and 8.5h, habitual bedtimes between 2200h and 0000h, and habitual morning awakenings between 0600h and 0900h; sleep-wake measures were confirmed objectively using actigraphy. They had no evidence of habitual

napping, no sleep disturbances (i.e., no complaints of insomnia, daytime sleepiness, or other sleep-wake disturbances), and neither extreme morningness nor extreme eveningness, as assessed by questionnaire (Smith et al., 1989). Subjects were free of acute and chronic medical and psychological conditions, as established by interviews, clinical history, questionnaires, physical examinations, and blood (including a fasting blood glucose test) and urine tests. Subjects were nonsmokers and did not participate in shift work, transmeridian travel, or irregular sleep/wake routines in the 60 days prior to the study. Enrolled subjects were monitored at home with actigraphy, sleep-wake diaries, and time-stamped call-ins to assess bedtime and waketime during the week prior to the in-laboratory phase and the week after the laboratory phase. Subjects were not permitted to use caffeine, alcohol, tobacco, and medications (except oral contraceptives) in the week before the laboratory experiment, as verified by urine screenings. Sleep disorders were excluded by a night of laboratory polysomnography and oximetry measurements.

The research protocols were approved by the Institutional Review Board of the University of Pennsylvania. All subjects provided written informed consent before enrollment and were compensated for their participation.

Experimental Design

Subjects participated in a protocol in the Sleep and Chronobiology Laboratory at the Hospital of the University of Pennsylvania and were studied for 14 consecutive days continuously with daily clinical checks of vital signs and symptoms by nurses (with an independent physician on call). During the protocol, subjects experienced five consecutive nights of sleep restricted to 4h TIB per night (0400h–0800h). This dose of sleep restriction was selected because it produces cumulative neurobehavioral deficits in most healthy adults (Van Dongen et al., 2003) and is within the range of sleep loss that occurs as a result of lifestyle factors (Knutson et al., 2010; Luckhaupt et al., 2010). Protocol days are labeled as follows throughout the manuscript: extended wakefulness (EW, the day following a night of 12h TIB with a 0400h bedtime); sleep restriction

days 1–4 (SR1–4, days following 4h TIB with a 0400h bedtime); sleep restriction day 5 (SR5, the fifth day following 4h TIB with a 2200h bedtime, **Figure 5.1**).

During the in-laboratory phase of the study, subjects were not permitted to leave the laboratory. Subjects were ambulatory and were permitted to watch television, read, play video or board games, and perform other sedentary activities between test bouts (which were completed while sitting at a computer) but were not allowed to exercise. Subjects wore a wrist actigraph throughout the in-laboratory protocol. On certain protocol days, subjects wore ambulatory electroencephalography (EEG) and electrocardiography (ECG) recording equipment for 24-h intervals. The light levels in the laboratory were held constant at < 50 lux during scheduled wakefulness and < 1 lux during scheduled sleep periods. Ambient temperature was maintained between 22°- 24°C. Subjects were behaviorally monitored by trained staff continuously throughout the protocol to ensure adherence.

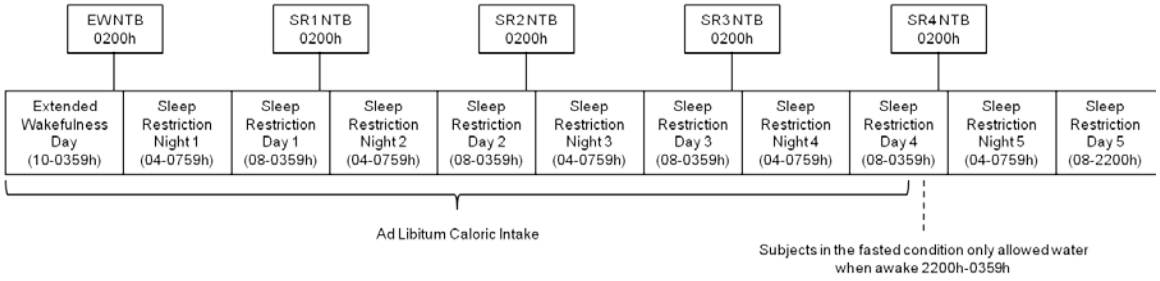

FIGURE 5.1: Protocol Schedule

Protocol days are labeled as follows: extended wakefulness (EW, the day following a night of 12h TIB with a 0400h bedtime); sleep restriction days 1–4 (SR1–4, days following 4h TIB with a 0400h bedtime); and sleep restriction day 5 (SR5, the fifth day following 4h TIB with a 2200h bedtime). For all subjects, food/drink was ad libitum for all days of the study except from 2200h-0359h on the fourth day following sleep restriction, when subjects in the fasted condition were only allowed water. Subjects completed a 30-minute computerized Neurobehavioral Test Battery (NTB) every 2 hours during scheduled wakefulness; only NTB performance during 0200h on each night of sleep restriction was used for analyses.

Measures

Caloric Intake

Subjects selected their meals/snacks by choosing from various menu options, selecting additional food/drink available in the kitchen within the laboratory suite (which included a refrigerator, microwave, and toaster oven) and by making requests to the study staff. To ensure subjects were provided sufficient time to eat each day, three 30- to 45-min eating opportunities were specified in the protocol during days with a 2200h bedtime (0900h, 1235h, and 1830h) and one additional 30-min opportunity to eat was specified in the protocol during days with a 0400h bedtime (0030h). In addition to these specified meal times, subjects were allowed to consume food/drink at any time during the protocol other than when they were completing neurobehavioral tests or sleeping. Subjects could eat what they pre-ordered through menus or could select from other foods available in the laboratory kitchen and could eat as much (or as little) as they wanted. Subjects retrieved their own food/drink from the laboratory kitchen when they wanted to eat/drink and could eat at a table in the common area or privately in their bedrooms.

All food was weighed and recorded prior to being available to the subjects. To enhance the measurement accuracy of each food item's weight, items were provided in individual containers. Each day, a detailed description of the items and the amount consumed and intake time were recorded by trained monitors. Additionally, any food/drink that was left over after each meal was weighed and recorded. The intake data were entered into The Food Processor SQL program (ESHA Research, Salem, OR), a validated (Hise et al., 2002) professional nutrition analysis software and database program that provides components of food/drink intake including calories and macronutrients.

Food/drink was ad libitum for all days of the protocol except the fourth day following sleep restriction. From 2200h-bedtime (0400h) on the fourth day following sleep restriction, subjects in the fasted condition were not allowed to consume any food/drink (besides water), whereas subjects in the fed condition were allowed to consume food/drink ad libitum (**Figure 5.1**).

Neurobehavioral Performance

Subjects completed a 30-minute computerized Neurobehavioral Test Battery (NTB) every 2 hours during scheduled wakefulness. During sleep restriction, NTBs occurred during the following times: 0800h, 1000h, 1200h, 1400h, 1600h, 1800h, 20000h, 2200h, 0000h and 0200h. Only NTB performance during 0200h on each night of sleep restriction was used for analyses (**Figure 5.1**). The 0200h NTB was chosen as the outcome measure for two reasons. Neurobehavioral performance is particularly impaired at 0200h due to sleep homeostatic and circadian factors (Goel et al., 2013). In addition, physiological responses to the ingestion of food (increased glucose levels, mechanical and chemical digestion, gastric emptying) are completed within approximately 4 hours of eating/drinking (Kong and Singh, 2008; Monnier and Colette, 2009); therefore, even if subjects in the fasted condition consumed food/drink right before the fasting period began (2200h), they were no longer experiencing postprandial effects at the time of testing. The NTB included various tests of sustained attention, working memory and cognitive throughput as well as subjective measures of sleepiness, fatigue, mood and stress.

The Psychomotor Vigilance Test (PVT), a 10-minute sustained-attention reaction time task with a random inter-stimulus interval of 2-10 seconds, is free of aptitude and learning effects and is widely used in sleep studies due to its sensitivity to sleep loss and functioning at an adverse circadian phase (Dinges and Powell, 1985; Lim and Dinges, 2008). Two primary outcome measures of the PVT that are particularly sensitive to sleep loss are reaction time (mean reciprocal reaction time, RRT = 1/ average reaction time (msec) X 1000; mean fastest reaction time, FRT = average fastest 10% of reaction times, msec) and errors of omission (Lapses = number of times reaction times > 500 msec) (Basner and Dinges, 2011). The digit span (DS) task (Wechsler, 1981), which requires subjects to remember a series of digits in either forward or reverse order, is a subject-paced task of working memory. The digit symbol substitution task (DSST) (Wechsler, 1981), which requires subjects to match a digit with a symbol, and the serial addition/subtraction task (SAST) (Thorne et al., 1985), which requires subjects to complete mental arithmetic questions, are subject-paced tasks of cognitive throughput. The primary

outcome measures for the DS, DSST and SAST are the total number of correct responses and performance on these tasks has been shown to be sensitive to sleep loss (Van Dongen et al., 2003; Goel et al., 2009a; Lim and Dinges, 2010).

The Karolinksa Sleepiness Scale (KSS), a 9-graded Likert scale (Åkerstedt and Gillberg, 1990), and a visual analog scale of fatigue (VAS-Fatigue), anchored by “fresh as a daisy” and “tired to death” (Monk, 1989), were used to assess subjective sleepiness and fatigue. The Profile of Mood States (POMS) was used to assess changes in mood. The POMS consists of a list of 37 adjectives, subjects must indicate the degree to which each adjective describes them at the moment using a 5-graded Likert scale. The primary outcome measures for the POMS are the scores for six subscales: Fatigue-Inertia, Vigor-Activity, Tension-Anxiety, Depression-Dejection, Anger-Hostility, and Confusion-Bewilderment (Shacham, 1983). Finally, a visual analog scale of stress (VAS-Stress) anchored by “not stressed at all” and “very stressed” was used to assess subjective levels of stress (Monk, 1989).

Statistical Analyses

Repeated-measures analysis of variance (ANOVA) compared 0200h neurobehavioral performance across sleep restriction days (EW-SR4). Multivariate ANOVAs compared 0200h neurobehavioral performance between subjects in the fed and fasted conditions on SR1-4. To account for individual differences in neurobehavioral performance, performance on EW (when subjects were kept awake until 0400h but were not sleep restricted during the prior night) for each assessment, was entered as a covariate in the multivariate ANOVAs (for example, EW DSST performance was a covariate when examining differences between fed and fasted subjects on SR1-4 DSST performance).

RESULTS

Subject Characteristics

Subjects in the fed condition (n = 20, 35.35 ± 10.23 y, 24.81 ± 3.00 BMI, 50% female) did not differ from subjects in the fasted condition (n = 24, 33.50 ± 8.37 y, 24.79 ± 3.47 BMI, 50% female) in age (P = 0.51), BMI (P = 0.98), the percentage of females (P =1.0) or estimated daily caloric need for energy balance (Harris-Benedict equation for basal metabolic rate multiplied by 1.4 to reflect the low physical activity levels of the laboratory (Harris and Benedict, 1918; Black, 1996) (P = 0.17). On SR4, all subjects except 1 (n=19 / 20) in the fed condition consumed calories (492.17 ± (SD) 307.68 kcal) from 2200h-0159h (the time before the 0200h NTB when subjects in the fasted condition were only allowed to consume water).

Impact of Sleep Restriction on Neurobehavioral Performance

Performance on the PVT, DSST and SAST varied significantly across days (EW-SR4) showing a linear decline as sleep restriction days progressed (PVT RRT: F (4, 172) = 16.61, P < 0.001; PVT FRT: F (4, 172) = 10.91, P < 0.001; PVT Lapses: F (4, 172) = 6.45, P < 0.001; DSST: F (4, 172) = 4.13, P = 0.003; SAST: F (4, 172) = 3.32, P = 0.012; linear contrasts all P < 0.01); performance on the DS did not vary significantly across days (P = 0.42) (**Figure 5.2**).

Subjective ratings of sleepiness and stress varied significantly across days (EW-SR4) showing a linear increase as sleep restriction days progressed (KSS: F (4, 172) = 19.83, P < 0.001; VAS-Fatigue: F (4, 172) = 7.49, P < 0.001; VAS-Stress: F (4, 172) = 4.19, P = 0.003; linear contrast all P < 0.01, **Table 5.1**). Ratings on the POMS subscales Tension-Anxiety, Fatigue-Inertia, Confusion-Bewilderment, and Vigor-Activity varied significantly across days (EW-SR4). Ratings of Tension-Anxiety (F (4, 172) = 2.65, P = 0.035), Fatigue-Inertia (F (4, 172) = 16.38, P < 0.001), and Confusion-Bewilderment (F (4, 172) = 5.23, P = 0.001) linearly increased as sleep restriction days progressed (linear contrasts all P < 0.05) while ratings of Vigor-Activity linearly decreased (F (4, 172) = 3.59, P = 0.008, linear contrast P = 0.017) (**Table 5.1**). Ratings of Depression-Dejection (P = 0.08) and Anger-Hostility (P = 0.11) did not change during sleep restriction (**Table 5.1**).

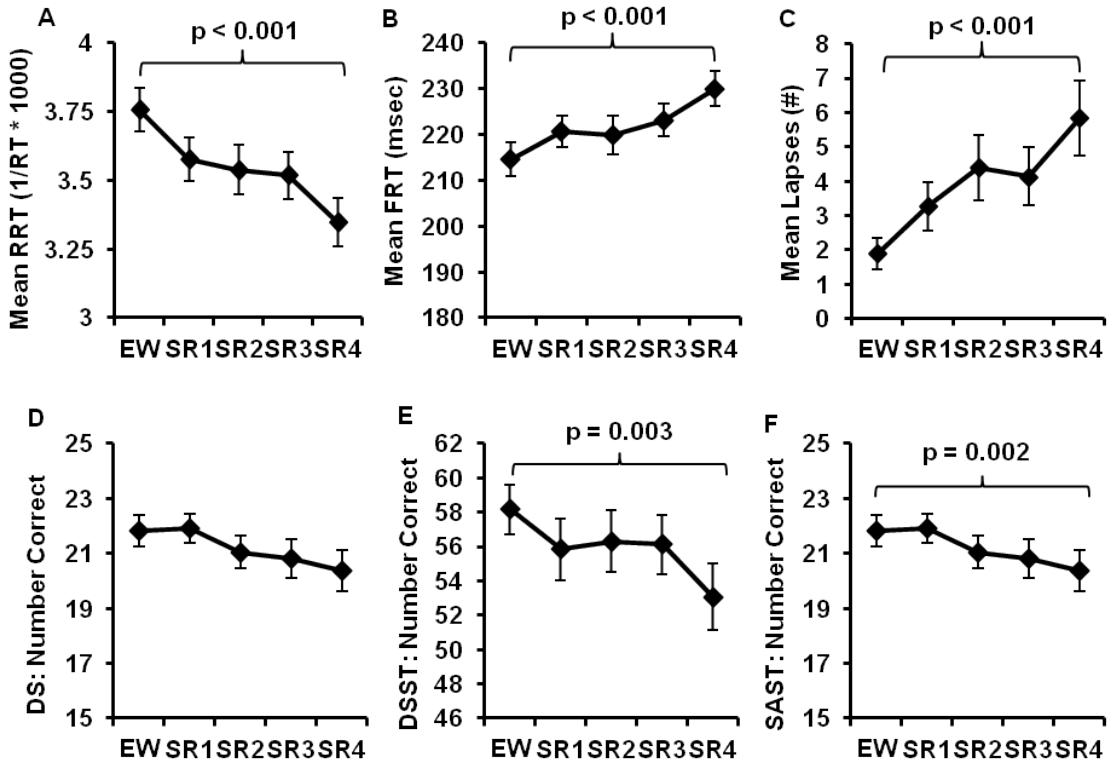

FIGURE 5.2: Neurobehavioral Performance at 0200h across Days of Sleep Restriction

The Psychomotor Vigilance Test (PVT) is a sustained-attention reaction time task; three primary outcome measures of the PVT are reaction time errors of omission (**A**: mean reciprocal reaction time, RRT = 1/ average reaction time, msec X 1000; **B**: mean fastest reaction time, FRT = average fastest 10% of reaction times, msec and **C**: Lapses = number of times reaction time > 500 msec). Performance on each of these measures showed a linear decline across sleep restriction days; note that for RRT (**A**), lower scores represent worse performance whereas for FRT (**B**) and Lapses (**C**), higher scores represent worse performance. The digit symbol substitution task (DSST) and Digit Span (DS) task are tasks of working memory and the serial addition/subtraction task (SAST) measures cognitive throughput. The primary outcome measures for the DS (working memory), DSST and SAST (cognitive throughput) are the total number of correct responses. Performance on the DSST (**E**) and SAST (**F**) showed a linear decline across sleep restriction days. Performance on the DS (**D**) did not vary significantly across days. Data expressed as mean ± SEM.

TABLE 5.1. Mean ± SD subjective measures of sleepiness, fatigue, stress and mood at 0200h across sleep restriction days

	EW	SR1	SR2	SR3	SR4
KSS[a]	5.73 ± 2.41	7.16 ± 2.33	7.07 ± 2.46	6.89 ± 2.71	7.59 ± 2.37
VAS-Fatigue[a]	50.77 ± 29.93	58.68 ± 29.27	65.25 ± 30.46	64.41 ± 30.20	69.00 ± 29.41
VAS-Stress[a]	16.05 ± 21.74	17.16 ± 24.15	22.73 ± 24.39	26.89 ± 27.24	25.39 ± 29.82
POMS					
Tension-Anxiety[a]	0.89 ± 1.51	0.93 ± 1.47	0.95 ± 1.72	1.48 ± 2.52	1.50 ± 2.28
Fatigue-Inertia[a]	3.86 ± 4.37	6.55 ± 5.74	7.88 ± 5.93	7.93 ± 6.02	8.68 ± 6.09
Confusion-Bewilderment[a]	0.84 ± 1.40	1.68 ± 1.91	1.55 ± 2.07	2.22 ± 2.77	2.022 ± 2.77
Vigor-Activity[a]	3.98 ± 5.84	2.75 ± 5.35	2.45 ± 5.65	2.80 ± 5.75	2.39 ± 4.91
Depression-Dejection	0.07 ± 0.25	0.55 ± 1.32	0.41 ± 1.19	0.73 ± 2.32	0.57 ± 1.66
Anger-Hostility	0.43 ± 1.15	0.45 ± 1.13	0.43 ± 0.97	0.80 ± 1.46	0.75 ± 1.38

[a] Significant linear change across sleep restriction days (all $P < 0.05$)

Impact of Fasted or Fed Condition on Neurobehavioral Performance

Only neurobehavioral measures that showed a significant effect of sleep restriction were used in the analyses comparing subjects in the fed and fasted conditions. During the 0200h NTB on SR 1, 2 and 3, when all subjects were fed ad libitum, performance on the PVT (RRT: all $P > 0.58$, FRT: all $P > 0.46$, Lapses: all $P > 0.53$), DSST (all $P > 0.27$), and SAST (all $P > 0.06$) did not significantly differ between subjects in the fed and fasted conditions. During the 0200h NTB on SR4, when subjects in the fed condition were allowed to consume food/drink ad libitum and subjects in the fasted condition were only allowed to consume water from 2200h until bedtime, performance on the DSST ($P = 0.47$) and SAST ($P = 0.40$) did not differ between condition

groups. However, fasted subjects performed better than fed subjects on the PVT (RRT: F (1, 41) = 3.48, *P* = 0.069, FRT: F (1, 41) = 5.58, *P* = 0.023, Lapses: F (1, 41) = 4.39, *P* = 0.042), **Figure 5.3**. In addition, repeated measures ANOVAs comparing PVT performance on SR3 and SR4 revealed that fed subjects exhibited significantly slower reaction times on SR4 compared to SR3 (RRT: F (1, 19) = 11.01, *P* = 0.004, FRT: F (1, 19) = 8.73, *P* = 0.008) and more lapses (F (1, 19) = 6.38, *P* = 0.021) whereas fasted subjects did not show this decline in performance (RRT: *P* = 0.13, FRT: *P* = 0.18, Lapses: *P* = 0.93), **Figure 5.3**. Conversely, there were no differences between fed and fasted subjects when examining subjective ratings of sleepiness, fatigue, stress, and mood on SR4 (all *P* > 0.15).

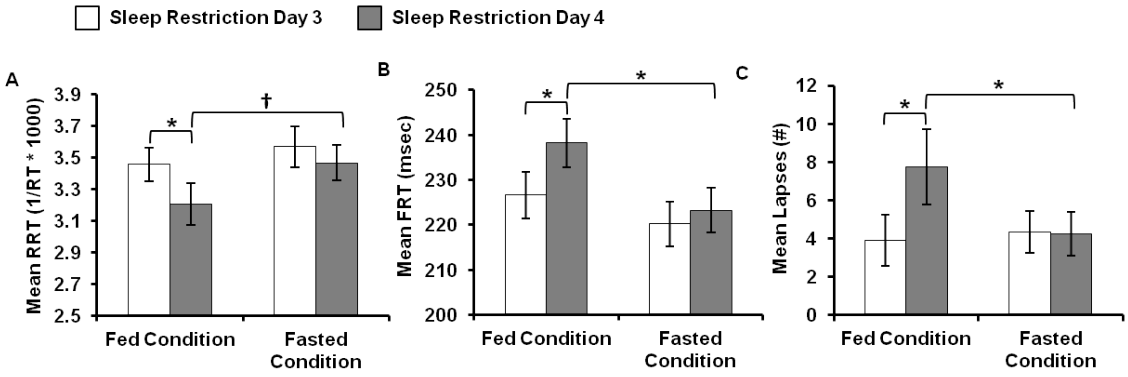

FIGURE 5.3: Change in Neurobehavioral Performance from Sleep Restriction Day 3 to Sleep Restriction Day 4 in Fed and Fasted Subjects

During the 0200h NTB on sleep restriction days 1, 2 and 3 (SR1-3), when all subjects were fed ad libitum, performance on the PVT did not significantly differ between subjects in the fed and fasted conditions. During the 0200h NTB on the fourth day following sleep restriction (SR4), when subjects in the fed condition were allowed to consume food/drink ad libitum and subjects in the fasted condition were only allowed to consume water from 2200h-bedtime, fasted subjects performed better than fed subjects on the PVT (**A, B** and **C**, †*P* = 0.069, *****all *P* < 0.05). In addition, subjects in the fed condition exhibited slower reaction times (**A** and **B**) and more lapses (**C**) on

the fourth day of sleep restriction compared to the third day of sleep restriction (*****all $P < 0.05$) whereas subjects in the fasted subjects did not show a significant decline in performance. Note that for RRT (**A**), lower scores represent worse performance whereas for FRT (**B**) and Lapses (**C**), higher scores represent worse performance; data expressed as mean ± SEM.

DISCUSSION

The aim of the current study was to assess if refraining from consuming late-night calories would affect neurobehavioral performance during sleep restriction. On the fourth day following sleep restriction at 0200h, a time when performance is particularly impaired due to sleep homeostatic and circadian factors, subjects who were fasted from 2200h until bedtime performed better on the Psychomotor Vigilance Test (PVT), an assessment of sustained attention, than subjects who were fed ad libitum during that time. In addition, subjects in the fasted condition did not show the further decline in performance from the third to fourth days following sleep restriction that was displayed by subjects in the fed condition. These results indicate that refraining from eating during late-night hours may be a strategy that can be used to alleviate decreased vigilance when sleep deprived.

Vigilance refers to the ability to maintain tonic alertness and sustain attention to a task over an extended period of time (Oken et al., 2006). Arousal and wakefulness, which refer to non-specific activation of the cerebral cortex, are necessary aspects of vigilance (Oken et al., 2006). The PVT, a task that requires subjects to attend to an intrinsically non-rewarding stimulus that is randomly presented for 10 minutes, is a reliable assay of vigilance. We found that consecutive nights of sleep restriction caused a slowing of reaction times (RRT and FRT) and an increase in the number of omission errors (Lapses) on the PVT which is consistent with previous studies assessing the effect of sleep loss on sustained attention performance (Lim and Dinges, 2008). Refraining from consuming calories during late-night hours on the fourth day following sleep restriction attenuated (but did not eliminate) this decline in vigilance caused by sleep restriction.

There were no differences between fed and fasted subjects on tasks of working memory or cognitive throughput. Tasks of working memory require subjects to maintain and/or manipulate relevant information for a short period of time before making a decision and responding and tasks of cognitive throughput require subjects to make repeated responses of a rehearsed process within a fixed period of time (Lim and Dinges, 2010). Thus, although working memory and cognitive throughput require attention, they do not involve vigilance. Although more research is needed to establish what aspects of neurobehavioral performance are affected by the fasted state, the results of this study suggest that the beneficial effects of refraining from late-night food/drink intake are specific to vigilance. Contrary to previous studies (Wells et al., 1995; Wells et al., 1997), we did not observe a difference between fed and fasted subjects on subjective measures of sleepiness, fatigue, stress or mood. Previous data from our laboratory show that subjective and objective measures of wakefulness/sleepiness are often unrelated; sleep-restricted individuals who exhibit profound impairments on the PVT may not rate their levels of sleepiness very highly whereas sleep-restricted individuals who exhibit very stable PVT performance may rate their sleepiness as increasingly high (Van Dongen et al., 2003; Van Dongen et al., 2004). Finally, although sleep restriction led to a small increase in self-reported stress levels, fasting at 2200h did not affect stress ratings when assessed at 0200h.

The current study supports the idea that sleep and energy balance are two interacting homeostatic systems with hunger associated with arousal and satiety associated with somnolence. It is believed that hunger increases vigilance in order to ensure that the organism will be alert in order to recognize opportunities to acquire food whereas satiety promotes sleep in order to conserve energy (Nicolaidis, 2006; Vanitallie, 2006). Indeed, rodent studies have shown that lack of food availability leads to increases in the duration of waking (Jacobs and McGinty, 1971; Danguir and Nicolaidis, 1979; Szentirmai et al., 2010) and a recent study demonstrated that flies who were sleep deprived due to starvation did not exhibit the learning impairments that flies who were sleep deprived using an automated apparatus displayed (Thimgan et al., 2010). Studies in humans also suggest that caloric intake, particularly fat intake, decreases alertness

(Craig and Richardson, 1989; Reyner et al., 2012; Smith and Miles, 1986a,b) whereas fasting increases visual attention to foods with hunger levels positively related to vigilance for certain types of foods (Castellanos et al., 2009; Gearhardt et al., 2012).

One underlying mechanism for this effect may be changes in the signaling of ligands that regulate energy balance and sleep/wake. Circulating levels of ghrelin, an orexigenic peptide produced in the stomach and proximal small intestine, increase prior to meal-taking and decrease rapidly after eating (Cummings et al., 2004, Callahan et al., 2004, Spiegel et al., 2011). Ghrelin levels also show a circadian rhythm with levels beginning to increase during late-night hours, peaking around 0200h and then decreasing as the night of sleep progresses (Cummings et al., 2001; Goel et al., 2009b, Spiegel et al., 2011). Under controlled feeding conditions, sleep restriction leads to increased ghrelin levels (Spiegel et al., 2004b, Schmid et al., 2008; Nedeltcheva et al., 2010). Although the effect of ghrelin administration on sleep/wake in humans is unclear (Garcia-Garcia et al., 2014), ghrelin administration promotes wakefulness in rodents (Szentirmai et al., 2006, Szentirmai et al., 2007).

Ghrelin may influence arousal by modulating the firing rate of orexin neurons. Orexin neurons, located in the lateral hypothalamic area, have widespread projections throughout the brain with particularly dense nerve endings in the tuberomammillary nucleus, paraventricular thalamic nucleus, arcuate nucleus of the hypothalamus and the locus ceruleus (Tsujino and Sakurai, 2013). Orexin neurons play a vital role in regulating the ‘flip-flop’ circuit that promotes either wake or sleep states; specifically, orexin activation increases the strength of the arousal system (Saper, 2006). Several ligands related to energy balance, including ghrelin, cholecystokinin, glucogon-like peptide 1, glucose, neuropeptides Y, and leptin, influence the firing rate and the membrane potential of orexin neurons (Tsujino and Sakurai, 2013). In rodents ghrelin administration excites orexin neurons (Yamanaka et al., 2003) whereas glucose inhibits orexin neurons (Tsujino and Sakurai, 2013).

Therefore, it is possible that, during late-night hours on the fourth day following sleep restriction, short-term fasting led to an increase in ghrelin which excited orexin neurons and

promoted wakefulness and improved PVT performance, whereas ad libitum feeding led to a rapid decrease in ghrelin and increase in glucose which inhibited orexin neurons and promoted sleepiness and impaired PVT performance. Future studies are needed in humans to examine the relationship between food intake and vigilance under rested and sleep deprived conditions and to measure how levels of ligands involved in energy balance, such as ghrelin, leptin (Sinton et al., 1999) and neuropeptide Y (Ida et al., 1999; Szentirmai and Krueger, 2006), change during sleep restriction and relate to neurobehavioral performance.

Limitations

This study has the following limitations. First, although sleep was assessed using polysomnography during the first and fifth nights of sleep restriction, the data were not completely scored and could not be included in this chapter. We found that fasting from 0200h until bedtime increased vigilance; therefore, one would expect that sleep latency may be longer for fasted subjects. However, one study showed that nocturnal fat intake (assessed using a food diary completed by the subjects) was associated with decreased sleep efficiency and increased sleep latency (Crispim et al., 2011). Future studies are needed to examine how refraining from eating/drinking during late-night hours influences sleep timing, duration and quality under non-sleep deprived and sleep-deprived laboratory conditions. Second, our subjects were healthy, were between the ages of 22–50 y, and had BMIs within the range of 19–30. The results may therefore not generalize to other groups, including obese individuals, adolescents or the elderly. Assessing differences in how short-term fasting influences PVT performance during late-night hours between lean and obese subjects would be particularly interesting given that in rodents, food deprivation decreased sleep in lean but not obese rats (Danguir and Nicolaidis, 1979).

Conclusions

Approximately one-third of U.S. adults self-report habitually sleeping less than 7 hours per night (Schoenborn and Adams, 2010) and nearly 20% of employees work an evening- or night-shift (McMenamin, 2007). Due to the high prevalence of chronic partial sleep deprivation

caused by habitual short sleep, as well as the large number of jobs requiring adults to be awake and functioning during late-night hours, countermeasures aimed at reducing the impairments in neurobehavioral performance caused by sleep/wake and circadian factors are needed. Vigilance may be a particularly important aspect of performance to target since sustained attention and fast reaction times are required for many jobs that involve sleep loss and shift-work such as those in the fields of medicine, transportation and security. Using psychoactive stimulants (e.g. caffeine) and taking brief naps are commonly used to combat increased sleepiness/decreased alertness and have been shown to improve sustained attention performance (Spaeth et al., 2014b). However, stimulants can lead to negative side effects, such as increased heart rate and blood pressure and naps can lead to impaired performance upon awakening due to sleep inertia (Spaeth et al., 2014b). Therefore, there is an important need to identify new countermeasures. Refraining from eating during late-night hours may represent one strategy for attenuating (but not eliminating) reduced vigilance when sleep deprived.

ACKNOWLEDGMENTS

This research was supported by NIH grant F31 AG044102 (AMS); the Department of the Navy, Office of Naval Research Award No. N00014-11-1-0361 (NG); and Clinical and Translational Research Center (CTRC) grant UL1TR000003. The authors thank the subjects who participated in the experiments and the faculty and staff of the Division of Sleep and Chronobiology who helped acquire the data.

CHAPTER 6: DISCUSSION

Consistent with the model proposed in the introduction (**Figure 1.2**), in a controlled laboratory environment with ad libitum food/drink access, restricting sleep to 4 hours per night for 5 consecutive nights led to weight gain, increased caloric intake and decreased energy expenditure. The increase in daily caloric intake was primarily due to the consumption of additional calories during late-night hours of extended wakefulness. This late-night eating may be detrimental to maintaining vigilant attention when sleep deprived – compared to those who consumed food/drink ad libitum, subjects who were fasted exhibited improved performance on the Psychomotor Vigilance Test during late-night hours. The summary and time-course of these findings are presented in **Figure 6.1**. Men and African Americans are more vulnerable to the weight gain effects of sleep restriction than women and Caucasians, respectively. During sleep restriction, men gained more weight, increased daily caloric intake to a greater degree, and consumed more calories during late-night hours of additional wakefulness than women but exhibited similar levels of energy expenditure. Relative to Caucasians, African Americans exhibited a similar increase in daily caloric intake during sleep restriction, consumed a comparable amount of calories during late-night hours of additional wakefulness, but exhibited greater deficits in energy expenditure throughout the study.

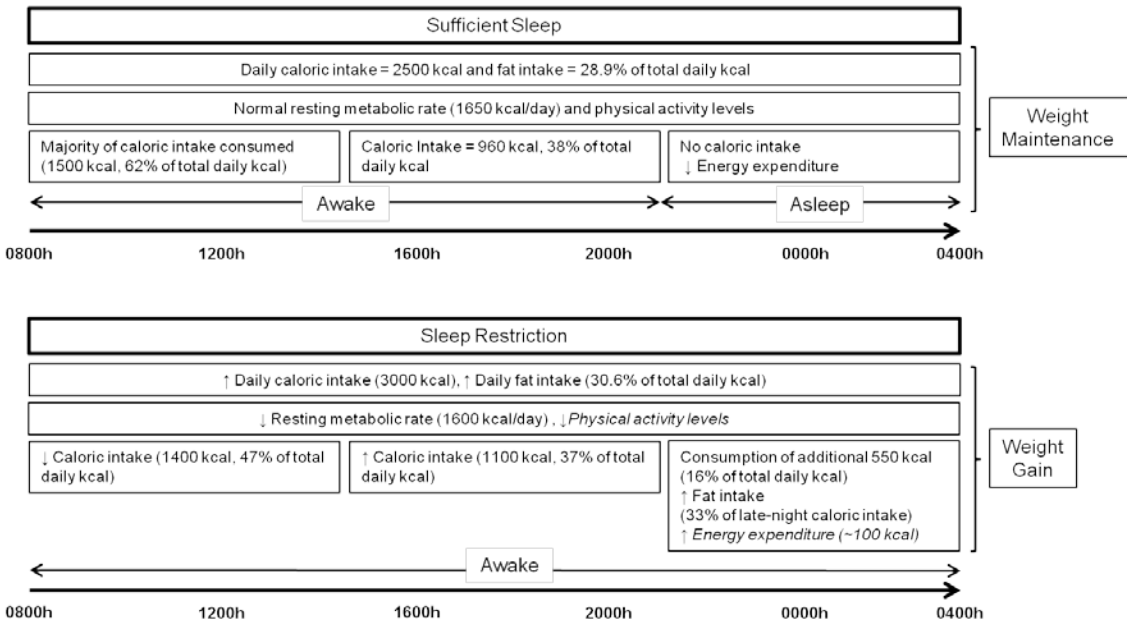

FIGURE 6.1: Depiction of how Sleep Restriction affects Energy Balance

This illustration summarizes the time-course of how sleep restriction affects energy intake and energy expenditure to promote weight gain. Results reflect the mean changes from results of this dissertation. Data from other studies are presented in *italicized* font (daily physical activity levels and energy expenditure during late-night hours).

SUMMARY OF MAIN FINDINGS

The finding that experimental sleep restriction leads to weight gain (dissertation chapter 2: 0.97 ± (SD) 1.4 kg; dissertation chapter 3: 1.12 ± (SD) 1.34) is consistent with a recent laboratory study showing that chronic sleep restriction (5h sleep/night for 5 nights) produced 0.82 ± (SD) 0.47 kg weight gain in a small sample of young adults (Markwald et al., 2013) and provides support for cross-sectional and prospective studies indicating a significant association between sleep duration and weight gain (Appelhans et al., 2013; Chaput et al., 2008; Lyytikainen et al., 2011; Mozaffarian et al., 2011; Xiao et al., 2013). Epidemiological studies cannot determine directionality in order to show that sleep restriction *causes* weight gain. For example, it is possible that the state of positive energy balance/weight gain leads to shortened sleep duration. The

results from this dissertation show that healthy, weight-stable adults randomized to a sleep restriction condition exhibited significant weight gain whereas individuals randomized to a control condition did not exhibit significant weight gain. Thus, these results provide evidence that sleep restriction *causes* weight gain. Interestingly, Markwald and colleagues (2013) found that spending 9 hours in bed for 5 additional nights prior to experiencing sleep restriction significantly attenuated the weight gain response. This is consistent with the beneficial effects of "banking sleep" (spending 10 hours in bed for 7 nights) on neurobehavioral performance during sleep restriction (Rupp et al., 2009). Future studies are needed to better understand how preemptively extending sleep impacts the neurobehavioral and energy balance responses to sleep restriction.

It has now been consistently shown that insufficient sleep increases daily caloric intake (Bosy-Westphal et al., 2008; Nedeltcheva et al., 2009b; Brondel et al., 2010; St-Onge et al., 2011; Calvin et al., 2013; Markwald et al., 2013; Spaeth et al., 2013 [dissertation chapter 2]; Spaeth et al., 2014a [dissertation chapter 3]). This result is maintained when sleep is restricted to 4-5 hours during the middle of the night (Markwald et al., 2013) or the second half of the night (Spaeth, et al., 2013). However, it remains unknown if sleeping in the first half of the night and getting up early (e.g., 0200h) also leads to increased caloric intake and whether this differs between individuals with a morning or evening chronotype. Although extended wakefulness does require additional energy, the additional calories consumed (~500 kcal) (dissertation chapters 2 and 3) exceed the amount of calories needed (~100 kcal) (Markwald et al., 2013; Shechter et al., 2013). Several studies have shown that increased caloric intake during sleep restriction exceeds daily caloric need and is positively correlated with weight gain (Bosy-Westphal et al., 2008; Nedeltcheva et al., 2009b; Spaeth et al., 2013). Thus, it is likely that increased caloric intake is the primary behavioral mechanism underlying the relationship between sleep duration and weight gain/obesity risk.

It remains unclear whether sleep restriction leads to greater consumption of specific macronutrients. Data from this dissertation (chapters 2 and 3) show that sleep restriction leads to an increase in the consumption of fat, particularly during late-night hours. This is consistent with

laboratory studies (Schmid et al., 2009; Brondel et al., 2010; St-Onge et al., 2011) and epidemiological studies (Shi et al., 2008; Grandner et al., 2010a) in adults and an observational study in adolescents (Weiss et al., 2010). However, other studies have found that sleep loss is associated with a greater craving for foods high in carbohydrate content (Spiegel et al., 2004), and increased carbohydrate intake (Nedeltcheva et al., 2009b; Beebe et al., 2013; Markwald et al., 2013). Recent functional magnetic resonance imaging studies show that, in response to food images (relative to non-food images), neuronal activation is greater in areas related to reward (e.g. anterior cingulate cortex, amygdala, putamen, nucleus accumbens, insula) and is reduced in higher-order cortical evaluation regions after sleep loss (1 night of total sleep deprivation or 5 nights of sleep restriction) than after habitual sleep (Benedict et al., 2012; St-Onge et al., 2012a; Greer et al., 2013). In addition, the neuronal responses to unhealthy food images (relative to healthy food images) were greater in reward areas after sleep restriction than habitual sleep (St-Onge et al., 2014). Many unhealthy calorically-dense foods are high in carbohydrate and fat content which may explain why studies have observed increases in both macronutrients. Indeed, subjects consumed more calories from condiments, desserts and salty snacks during sleep restriction but did not increase the consumption of healthier foods such as salad, vegetables and fruit or meat, eggs and fish (Spaeth et al., 2014a, [dissertation chapter 3]). Sleep restriction provides additional wakefulness which allows adults more time to consume food and drink. The accessibility of calorically dense unhealthy foods in today's environment (Swinburn et al., 1999) and the changes in brain activation in response to food stimuli caused by insufficient sleep combine to promote the hedonic consumption of unhealthy food/drink.

Recent research has highlighted the critical contribution of meal timing to weight regulation (Allison et al., 2014; Garaulet and Gomez-Abellan, 2014). During sleep restriction, subjects consumed a large amount of calories during late-night hours of additional wakefulness and then consumed fewer calories the next morning/early afternoon (Spaeth et al., 2013 [dissertation chapter 2]; Spaeth et al., 2014a [dissertation chapter 3]). This is consistent with other laboratory studies showing an increase in caloric intake during evening and late-night hours when

sleep restricted (Nedeltcheva et al., 2009b; Markwald et al., 2013) and decreased consumption the following morning (Markwald et al., 2013). Thus, sleep restriction delays meal timing such that the majority of calories are consumed between early-evening and late-night hours rather than between morning and early afternoon hours. Altered meal timing may produce weight gain independently from the amount or content of caloric intake. Mice fed during the light cycle (when they are normally asleep) gained more weight than mice fed during the dark cycle, even when caloric consumption and physical activity did not differ (Arble et al., 2009; Fonken et al., 2010). In humans, adults who consumed ≥ 33% of daily calories in the evening were at greater risk for obesity (Wang et al., 2013) and delayed eating was predictive of less weight loss (Garaulet et al., 2013). A recent experimental weight-loss study randomized overweight and obese women to two isocaloric conditions: 'breakfast' (700 kcal breakfast, 500 kcal lunch, 200 kcal dinner) or 'dinner' (200 kcal breakfast, 500 kcal lunch, 700 kcal dinner) for 12 weeks and found that women in the 'breakfast' condition lost more weight and had a greater reduction in waist circumference than women in the dinner condition (Jakubowicz et al., 2013). Although this is a relatively new area of interest (especially in human research) and more research is needed to tease apart the effects of caloric intake/macronutrient content and meal timing on weight gain, sleep-restricted adults may gain weight due to delayed meal timing.

It has now been shown in several studies using whole-room indirect calorimetry that energy expenditure is greater when awake than when asleep; therefore, energy expenditure is greater during a day with limited (4-5 hours sleep time) or no sleep compared to a day with a 7-9 hours of sleep (Jung et al., 2011; Markwald et al., 2013; Shechter et al., 2013). However, it is less clear how a night of insufficient sleep impacts energy expenditure during the subsequent day. Total energy expenditure is the sum of: resting metabolic rate (energy needed for cell repair, immune activity, thermoregulation, etc.), diet-induced thermogenesis (heat production caused by the intake and digestion of food) and physical activity levels (muscular work required for all spontaneous and willful movements) (Leonard, 2012). Several studies have found no effect of sleep restriction on resting metabolic rate or diet-induced thermogenesis (Bosy-Westphal et al.,

2008; Nedeltcheva et al., 2009b; Shechter et al., 2013) when assessed during the day following consecutive nights of sleep restriction. Data from this dissertation (chapter 4) demonstrate that five nights of sleep restriction leads to decreased resting metabolic rate during the following day. This is consistent with laboratory studies measuring resting metabolic rate following three weeks of sleep restriction combined with circadian disruption in young healthy adults (Buxton et al., 2012), and two weeks of sleep restriction combined with caloric restriction and one night of total sleep deprivation in healthy young men (Benedict et al., 2011). Studies examining physical activity consistently show that sleep-restricted subjects display lower physical activity levels especially in vigorous activity (Schmid et al., 2009; St-Onge et al., 2011; Bromley et al., 2012). Although it is unclear why there are discrepant findings related to the effects of sleep loss on measures of energy expenditure, differences in study design likely play a role. For example, allowing subjects to: leave the laboratory to walk outside (Nedeltcheva, et al., 2009), consume caffeine daily during the study (Nedeltcheva, et al., 2009; St-Onge, et al., 2011), use a gym at their leisure (St-Onge, et al., 2011) and sleep at home rather than supervised in the laboratory (Bosy-Westphal, et al., 2008) may have confounded the assessment of resting metabolic rate in previous studies. Although more research is needed in this area, decreased energy expenditure during the day following sleep loss (reductions in resting metabolic rate and physical activity) may also play an important role in the relationship between sleep restriction and weight gain/obesity.

Consistent with population studies (Ko et al., 2007; Meyer et al., 2012; Yang et al., 2013), men gained more weight than women during a laboratory protocol involving sleep restriction (Spaeth, et al., 2013 [dissertation chapter 2]). Although men consumed more calories than women during baseline and sleep restriction conditions, they also exhibited a greater increase in caloric intake during sleep restriction and consumed a greater proportion of calories during late-night hours (Spaeth et al., 2014a [dissertation chapter 3]). There were no gender differences in measures of energy expenditure (resting metabolic rate, diet-induced thermogenesis, or respiratory quotient; co-varying fat-free mass) after baseline sleep, sleep restriction or recovery sleep (dissertation chapter 4). Whether gender differences exist in physical activity levels during

the day following sleep restriction or in the amount of additional energy required during extended wakefulness is unknown. Sleep-restricted men likely gain more weight than women due to greater daily caloric intake and late-night consumption.

Two recent population studies have shown that the relationship between short sleep duration and increased risk for obesity is stronger in African Americans than Caucasians (Donat et al., 2013; Grandner et al., 2014) and African Americans gained more weight than Caucasians during a laboratory protocol involving sleep restriction (Spaeth, et al., 2013 [dissertation chapter 2]). Data from this dissertation suggest that deficits in energy expenditure combined with similar levels of caloric intake may explain the increased vulnerability to weight gain in sleep-restricted African Americans. Relative to Caucasians, African Americans exhibited a lower resting metabolic rate after baseline sleep, sleep restriction and recovery sleep, a smaller rebound in resting metabolic rate after recovery sleep, and a higher respiratory quotient (indicating decreased lipid metabolism) after sleep restriction (chapter 4) but consumed a comparable amount of calories during all times of day (Spaeth et al., 2014a [dissertation chapter 3]). More research is needed to understand if there are also race differences in physical activity levels during the day following sleep restriction or in the amount of additional energy required during extended wakefulness.

Millions of adults, such as those in the transportation and security fields, work extended hours and/or evening- or night-shifts and these individuals are at increased risk for obesity and diseases related to obesity (Esquirol et al., 2009; McMenamin, 2007). Results from this dissertation show that these workers may consume additional calories during late-night hours and that these calories are higher in fat compared to calories consumed during other times of day (Spaeth et al., 2013 [dissertation chapter 2]). Refraining from consuming these late-night calories may not only promote the maintenance of a healthy weight but also improve vigilant attention. Subjects who were fasted during late-night hours displayed improved vigilant attention relative to subjects who were fed ad libitum on the fourth day following consecutive nights of sleep restriction (dissertation chapter 5). Vigilant attention is particularly important for jobs in the transportation and security fields and fasting during overnight shifts may improve performance

and prevent workplace accidents. Future research is needed to identify the timing and duration of a fast that is optimal for improving performance.

IMPLICATIONS OF RESEARCH

Collectively, these findings have important implications for public health. After remaining relatively stable from 1960-1980, the prevalence of obesity in the U.S. has increased dramatically in the past 30 years; the age-adjusted overall prevalence of obesity in the U.S. reached 34.9% in adults (20+ y) and 17% in youths (2-19 y) in 2012 (Ogden et al., 2014). Obesity is a risk factor for a variety of chronic conditions including depression, type 2 diabetes, hypertension, and high cholesterol (Malnick and Knobler, 2006; Lopresti and Drummond, 2013). Considering the significant physiological and psychological consequences of obesity, uncontrolled weight gain poses a serious problem in the U.S. As such, research has focused on understanding mechanisms underlying the recent increase in obesity prevalence. Reduced sleep duration has been consistently identified as a lifestyle factor associated with obesity (Bo et al., 2011; Cappuccio et al., 2008; Di Milia et al., 2013; Theorell-Haglow et al., 2012; Yiengprugsawan et al., 2012). Importantly, this relationship has also been observed in children and adolescents (Al-Hazzaa et al., 2012; Ekstedt et al., 2013; Magee et al., 2013; Mitchell et al., 2013; Moraleda-Cibrian and O'Brien, 2013; Pileggi et al., 2013; Suglia et al., 2013; Chen et al., 2014; Magee and Lee, 2014). Data from this dissertation demonstrate that sleep restriction causes weight gain, increased daily caloric intake, greater consumption of fat and late-night eating. These findings support the notion that promoting good sleep hygiene will be beneficial in maintaining a healthy weight. A clinical trial is currently ongoing to investigate if sleep extension is an effective and feasible strategy for weight-loss in obese adults (Cizza et al., 2010; Chaput and Tremblay, 2012). Informing parents about the importance of ensuring that children and adolescents get sufficient sleep and maintain a consistent bedtime may help to curb the increasing prevalence of overweight and obese youths. Education related to the importance of maintaining good sleep habits is particularly important for African Americans given they report a higher prevalence of

habitual short sleep duration (Singh, et al., 2005; Hale and Do, 2007; Whinnery et al., 2014) and exhibit energy expenditure deficits that promote weight gain. Data from this dissertation also highlight the importance of recovery sleep when sleep restriction is unavoidable. Caloric intake and resting metabolic rate returned to baseline levels after one night of 12 hours of recovery sleep (dissertation chapters 2 and 4). For individuals that must experience sleep loss due to work or school schedules and deadlines, making time for recovery sleep during the weekend represents one way to limit weight gain.

Results from this dissertation also have important implications for basic scientific research in the field of sleep and energy balance. Using animal models to assess the effect of sleep loss on neurobehavioral and physiological outcomes is immensely important for understanding the behavioral patterns observed in humans. For example, animal studies measuring or manipulating steroid hormones may be able to inform what causes the gender differences observed in the caloric intake response to sleep restriction (dissertation chapter 3). In addition, the observed increase in meal frequency without a change in meal size during sleep restriction suggests that sleep restriction affects postprandial and meal initiation signals rather than meal termination signals. This provides animal researchers with a basis for examining how circuitry related to the control of sleep/wake may interact with signals related to meal frequency such as ghrelin, hypothalamic dopamine and amylin (Cummings and Overduin, 2007; Lutz, 2006; Meguid et al., 2000). There are fundamental differences between humans and rodents in the energy expenditure response to sleep restriction. Rechtschaffen and colleagues published seminal work from 1989-1990 examining the effects of sleep deprivation (using the disk-over-water method) on energy balance in rats and found that sleep deprivation led to death (totally sleep-deprived rats died within 2-3, partially sleep-deprived rats died within 4-6 weeks), weight loss, hyperphagia, a large rise in energy expenditure and thermoregulatory changes (Rechtschaffen and Bergmann, 2002). Recently, Barf and colleagues attempted to better model what occurs in humans by placing rats on a repeated cycle of a 5-day period of sleep restriction followed by a 2-day period of sleep allowance (mimicking a typical work week and weekend) and

found that during the sleep restriction period rats exhibited hyperphagia and weight loss whereas during the recovery sleep period rats exhibited food intake at levels comparable to the control group but gained significantly more weight (Barf et al., 2012). Thus, although both animals and humans increase caloric intake during sleep restriction (and decrease caloric intake to control levels after recovery sleep), the additional energy expended during extended wakefulness appears to be much greater in rodents than humans which leads to differential effects on body weight. More research is needed to understand this species difference and examine the effects of sleep loss on energy balance in other species.

POSSIBLE NEURAL MECHANISMS UNDERLYING INTERACTIONS BETWEEN SLEEP/WAKE AND ENERGY BALANCE

One key site for cross-talk between circuitry related to sleep/wake and energy balance is the hypothalamus (Saper, 2006; Vanitallie, 2006). Seminal lesioning studies in rodents illustrated the importance of the hypothalamus in regulating body weight and subsequent experiments have shown how distinct hypothalamic nuclei, such as the articulate nucleus, paraventricular nucleus, lateral hypothlamaic area, dorsomedial nucleus and ventromedial nucleus affect energy intake and energy expenditure (Schneeberger et al., 2014). Similarly, the hypothalamus plays a critical role in the ascending arousal system (i.e., the monoaminergic pathway involving histaminergic neurons in the tuberomammillary nucleus of the caudal hypothalamus and peptidergic neurons in the lateral hypothalamus) and is the site of the ventrolateral preoptic nucleus (VLPO) which innervates and inhibits the ascending arousal system (Saper et al., 2005). There are many projections from the lateral hypothalamic area and the dorsomedial hypothalamic nucleus to the VLPO (Chou et al., 2002). The suprachiasmatic nucleus, which centrally controls circadian rhythms, is located in the hypothalamus and the dorsomedial hypothalamic nucleus is one proposed site for the food-entrainable circadian pacemaker in rodents (Mieda et al., 2006). The suprachiasmatic nucleus also provides input to orexin-producing neurons in the lateral hypothalamic area and has reciprocal connections with the ventromedial arcuate nucleus (Vanitallie, 2006).

Orexin (also called hypocretin), a neurotransmitter produced in the lateral hypothalamic area, has been implicated in the control of both sleep/wake patterns and energy balance (Sakurai, 2005). Orexin-producing neurons are localized to the lateral hypothalamic area and project widely throughout the brain to other areas of the hypothalamus with particularly dense nerve endings in the tuberomammillary nucleus, paraventricular thalamic nucleus, arcuate nucleus of the hypothalamus and the locus ceruleus (Tsujino and Sakurai, 2013). Orexin neurons play a critical role in regulating the switch between activation of the ascending arousal system (wake) and activation of the VLPO (sleep) (Saper, et al., 2005). Several ligands related to energy balance, including ghrelin, cholecystokinin, glucogon-like peptide 1, glucose, neuropeptides Y, and leptin, influence the firing rate and the membrane potential of orexin neurons (Tsujino and Sakurai, 2013). Orexin neurons are stimulated by ghrelin, a meal initiation signal, and inhibited by glucose and leptin, which increase after the consumption of a meal (Cummings and Overduin, 2007; Tsujino and Sakurai, 2013). The orexin system also influences signals related to energy balance. For example, orexin stimulates appetite-promoting neuropeptide-Y neurons in the arcuate nucleus of the hypothalamus (Hanlon and Van Cauter, 2011; Tsujino and Sakurai, 2013) and orexin neurons have dense projections to the dopaminergic ventrotegmental area of the nucleus accumbens which is involved in the hedonic control of food intake (Korotkova et al., 2003). Finally, sleep deprivation leads to increased orexin activation in rats (Estabrooke et al., 2001; Martins et al., 2010), dogs (Wu et al., 2002) and monkeys (Zeitzer et al., 2007).

Collectively these studies indicate that orexin activation increases during sleep restriction to promote wakefulness and counteract sleep pressure which simultaneously promotes increased hedonic drive and greater caloric intake. After consuming food/drink, ghrelin rapidly decreases while levels of leptin and glucose increase (Cummings and Overduin, 2007) which inhibits orexin activity leading to decreased wakefulness. Consistent with this model (**Figure 6.2**), subjects increased caloric intake during sleep restriction (Spaeth et al., 2013 [dissertation chapter 2]; Spaeth et al., 2014a [dissertation chapter 3]) and subjects displayed decreased vigilant attention

after consuming food/drink ad libitum compared to subjects who refrained from eating during late-night hours (dissertation chapter 5).

Melanin-concentrating hormone (MCH) neurons are localized with orexin in the lateral hypothalamic area. MCH neurons influence sleep/wake by promoting sleep and stabilizing rapid-eye-movement sleep (Fraigne and Peever, 2013; Monti et al., 2013). In addition, MCH neurons are affected by metabolic status (Rolls et al., 2010; Barson et al., 2013) and MCH signaling promotes increased caloric intake and energy conservation. It is unclear how sleep deprivation affects MCH signaling; however, these neurons may represent another site of integration for sleep/wake and energy balance systems.

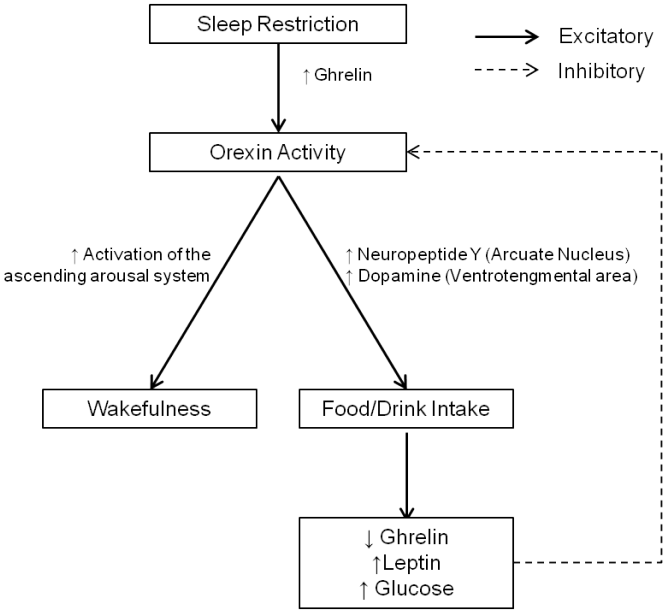

FIGURE 6.2: Diagram Illustrating how Orexin Influences the Sleep/Wake system and Energy Balance

Stress

The stress response, which interacts with both sleep/wake and energy balance systems, may also play role in the observed effects of sleep restriction on energy intake. Stress, defined as a threat (real or implied) to the psychological and/or physiological integrity of an individual, leads

to physiological and behavioral responses aimed at achieving homeostasis (McEwen, 2000). These responses can cause changes in the duration and quality of sleep as well as in caloric intake and metabolism (Rolls et al., 2010). A hallmark of the stress response is the activation of the hypothalamic-pituitary-adrenal (HPA) axis which involves the release of corticotrophin-releasing factor from the paraventricular hypothalamic nucleus which stimulates the release of adrenocorticotropic hormone form the pituitary which activates the release of cortisol from the adrenal cortex.

Exposure to stress and the subsequent activation of the HPA axis influences food-intake regulation in animals and humans and orexin neurons partially mediate this relationship (Bazhan and Zelena, 2013). Stress exposure and hormones involved in the HPA axis have been associated with a lack-of-appetite, decreased food intake and weight loss; however, stressed animals and humans will increase caloric intake if foods high in fat and sugar content are available (Bazhan and Zelena, 2013). It has been suggested that ingestion of palatable foods, a pleasurable activity, is a strategy used to reduce the discomfort of stress (Bazhan and Zelena, 2013).

Although sleep restriction produces a small but significant increase in subjective levels of stress (dissertation chapter 5; Dinges et al., 1997), most laboratory studies have found that acute and chronic sleep restriction have no effect on daily profiles of cortisol levels (Nedeltcheva, et al., 2009a; van Leeuwen et al., 2009; Leproult and Van Cauter, 2011; Schmid et al., 2011; Voderholzer et al., 2012; Axelsson et al., 2013; Pejovic et al., 2013); however, sleep restriction may lead to a transient increase in cortisol levels during afternoon/evening hours (Spiegel et al., 2004a; Buxton et al., 2010; Omisade et al., 2010; Reynolds et al., 2012). Although the majority of these studies were conducted in young, healthy, lean men and more research is needed to generalize the findings, these results suggest that sleep restriction in the laboratory does not significantly activate the HPA axis. Similarly, Rechtshaffen dismissed the stress explanation for the energy balance responses he observed in sleep-deprived rats because they did not exhibit

classic stress indicators such as stomach ulcers, adrenal hypertrophy, or increases in adrenocorticotropic hormone or corticosterone (Rechtschaffen and Bergmann, 2002).

There is evidence that sleep loss potentiates the stress response to psychosocial stressors; healthy adults randomized to a total sleep deprivation condition exhibited greater subjective stress, anxiety and anger in response to mild stressors than adults who slept (Minkel et al., 2012). Outside of the laboratory, insufficient sleep is often coupled with stress due to work or school obligations. More research is needed to understand how insufficient sleep interacts with the stress system and if HPA axis activation and/or subjective levels of stress moderate the effects of sleep loss on daily caloric intake, macronutrient content or meal timing.

LIMITATIONS

The studies included in this dissertation have several limitations. First, physical activity is an important factor that might contribute to weight gain. Subjects in our study lived within the confines of the laboratory suite and were not allowed to exercise during the protocol; therefore, activity levels were limited. Second, although caloric intake was ad libitum, subjects were only allowed to consume food and drink provided by hospital and laboratory staff; foods that contained caffeine (including chocolate) were prohibited. In addition, although there were approximately 10 opportunities to eat during a typical protocol day, subjects were not allowed to eat/drink during neurobehavioral testing that occurred throughout the day. Therefore, subjects may have desired to eat certain foods that were unavailable to them or may have wanted to eat during certain times when they were not allowed to do so due to testing; both factors may have reduced total caloric intake and subsequent weight gain. Third, subjects were healthy, were between the ages of 21–50 y, and had BMIs between the range of 19–30. The results may therefore

not generalize to other groups, including obese individuals, adolescents or the elderly. Finally, sleep restriction consisting of 4 hours per night for five consecutive nights was selected for these studies because this degree of sleep loss produces cumulative neurobehavioral deficits in most healthy adults (Van Dongen et al., 2003) and is within the range of sleep loss that occurs as a result of lifestyle factors; approximately 7.8% of adults report sleeping less than 5 hours per night (Krueger and Friedman, 2009). However, further research is needed to examine how more commonly experienced sleep restriction (20.5% of adults report sleeping 6 hours per night) affects weight gain and measures of energy balance.

FUTURE DIRECTIONS

The results of this dissertation not only provide important information needed to understand the effects of sleep duration on energy balance but also prompt future studies in this area of research. Although I have demonstrated that sleep restriction leads to weight gain, increased caloric intake and late-night eating, not all subjects responded to the same degree (e.g., some gained a significant amount of weight while others maintained or lost weight). I am currently examining if the caloric-intake responses to sleep restriction are stable over time within individuals. To do this, I am measuring caloric intake during two 5-day bouts of sleep restriction separated by either 1, 3 or 5 days of recovery sleep and or by several weeks. This will begin to elucidate whether or not the energy balance response to sleep restriction, like the neurobehavioral response (Van Dongen et al., 2004), is a stable trait-like characteristic. I am also interested in identifying biomarkers that underlie the individual differences observed in the energy balance response to sleep restriction. The amount of time spent in sleep stages 3 or 4 (slow-wave sleep) is stable within individuals but highly variable between individuals (including showing race and gender differences, reviewed in the dissertation introduction) and there is evidence that slow-wave sleep relates to energy balance (Shechter et al., 2012). Therefore, I am currently examining if slow-wave sleep duration (as well as other sleep parameters) relates to weight gain, daily caloric intake, macronutrient content and meal timing. Finally, I plan to examine how sleep

loss impacts changes in resting-state functional connectivity in areas of the brain related to energy balance in order to assess if these changes relate to caloric intake, macronutrient content and meal timing

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