Effects of Short-Term Sleep Restriction on Energy Balance in Healthy Young Adults

by

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Abstract

Insufficient sleep may be associated with obesity via increased energy intake and/or decreased energy expenditure. The present study therefore aimed to investigate effects of sleep restriction on energy balance in healthy young adults. Participants (14 men, 13 women) aged 35.3 $\pm$ 1.0 y with 23.6 $\pm$ 0.2 kg/m^2 BMI completed a randomized, crossover study exposed to short and habitual sleep with 4 wk washout. Controlled diets were provided during the first 4 d, followed by 2 d of ad libitum eating. Ad libitum energy intake, energy expenditure and physical activity level were determined as well as energy balance and body weight. Results showed that ad libitum energy intake (p = 0.031), as well as total fat (p = 0.018) increased after short compared with habitual sleep, but physical activity level, energy expenditure, energy balance, and body weight remained unaffected by sleep duration. In conclusion, sleep deprivation elevates energy intake, which may lead to positive energy balance over time and increase the risk of weight gain and/or obesity.

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Chapter 1: Introduction

Over the past few decades, there has been a ubiquitous growth in both overweight and obese populations. The drastic increase of these populations is particularly seen in developed countries. It was reported that the prevalence of overweight individuals among U.S. adults rose by 26% between 1976 and 2004, likewise the number of obese individuals increased from 15% in 1980 to 32% in 2004 (WHO, 2000; Ruhm, 2007). If this trend continues, more than 70% of U.S. adults will be either overweight or obese by 2020 (Ruhm, 2007).

Obesity is growing in its extreme form and has become an overwhelming public health issue (WHO, 2000; Magee *et al.*, 2010; Finer, 2011). It has been well documented that compared to non-obese individuals, obese people have much higher rates of morbidity and mortality attributed to chronic diseases such as cardiovascular disease, type 2 diabetes, and certain cancers (Finer, 2011; Mokdad *et al.*, 2001). As a consequence, the national cost spent on obesity-related disease is increasing rapidly. For example, it has been recently suggested that 6-10% of health care budgets are absorbed by obesity-related ill-health in European countries and North America (Lobstein, 2011). In addition to health concerns, obesity is likely to trigger psychological problems. For instance, overweight and obese people, suffering from social bias and discrimination, maybe have a higher propensity for inferiority complexes and low self esteem (WHO, 2000; Strauss, 2000).

In general, the fundamental causes of the overweight and obese condition can be categorized as involving genetic and non-genetic factors (WHO, 2000; Sun *et al.*, 2009). Apart from genetic factors such as parental obesity (Whitaker *et al.*, 1997), non-genetic

factors promote the construction of the so-called "obesogenic environment" in the modern world which is characterized by increased facilitation of hyperphagia with minimal procurement costs and sedentary lifestyles (Chaput *et al.*, 2010). Accordingly, research on prevention and treatment strategies for obesity over the last decade has concentrated on improving the environment and lifestyle.

On the road to anti-obesogenesis, a very important element of lifestyle and environment is sleep integrity. According to National Institutes of Health, most of adults need to obtain 7 to 9 h of sleep per night to function best, while sleeping 6 h or less is considered as short sleep (NIH, 2009). During the last few decades, short sleep is becoming prevalent. It has been recently estimated that more than 30% of Americans obtain only 6 h of sleep per night or less (Charlotte *et al.*, 2008) compared to 8-9 h of sleep in average over the past 50 yrs (Kripke *et al.*, 1979). Cumulative studies on the sleep-illness association have found that sleep deprivation, in particular, may relate to obesity by disturbing energy balance externally and/or internally as **Figure 1.1** shows**.** However, the association is still debatable given large differences in the target populations and the variety of studies (Cappuccio *et al.*, 2008). To date, a few clinical trials have directly measured the effect of sleep restriction on energy intake (Bosy-Westphal *et al*., 2008; Brondel *et al.*, 2010; Nedeltcheva *et al.*, 2009), concluding that acute sleep deprivation increases food and caloric intakes in healthy adults. However, whether short sleep affects energy expenditure is still controversial due to the existence of conflicting results. Therefore, the present study was proposed to assess how short-term sleep restriction affects energy balance in healthy young adults, particularly moderated

via changes in energy intake and expenditure. We hypothesized there would be no detectable impact of sleep restriction on energy balance and its components.

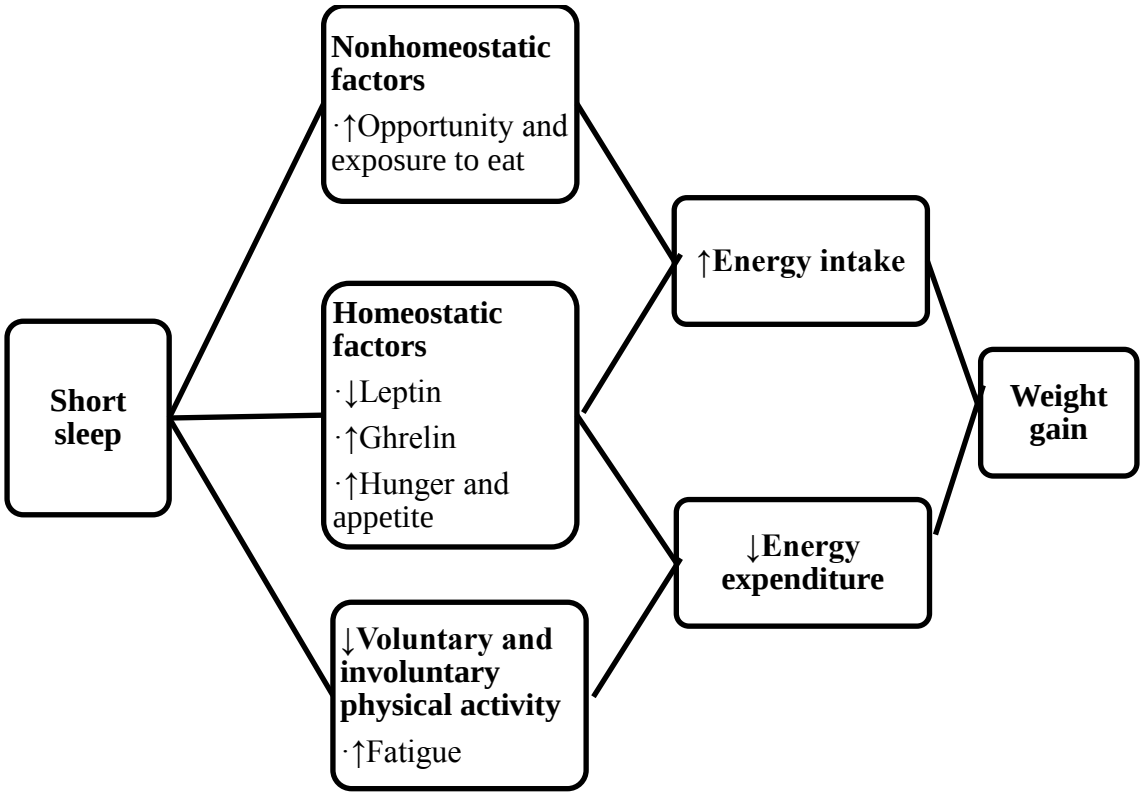

Figure 1.1 Potential mechanisms by which short sleep is associated with obesity (Knutson et al., 2007; Taheri, 2006; Gupta et al., 2010)

Chapter 2: Literature Review

2.1 Prevalence of Sleep Deprivation and Potential Risks

The prevalence of reduced sleep is increasing rapidly and globally year by year. In 1960, the average sleep duration was estimated to be 8.0-8.9 h which generally met human requirements (Kripke *et al.*, 1979), whereas the modal sleep duration in the past 10 years has dropped to less than 7 h/night according to the 2010 Sleep in America Poll conducted by the National Sleep Foundation (2010). In U.S., more than 3 in 10 American adults sleep 6 h/night or less based on the report published by National Centre for Health Statistics (Charlotte *et al.*, 2008). In 2007, a Canadian survey reported that residents in British Columbia only slept an average of 6.5 h/d (Fok *et al.*, 2007), which is not better than U.S. statistics. For children and adolescents, moreover, adequate sleep is particularly important for brain development and body growth, but their sleep duration was identified as less than required as well (Wolfson & Carskadon, 1998). According to Wolfson and Carskason (1998) who surveyed 3,120 high school students across ages 13-19, their average sleep time was 7.5 h during weekdays, which is 2 h less than the optimal sleep length for adolescents; additionally, 87% of students claimed daytime tiredness and 26% reported that they usually slept 6.5 h or less. If insufficient sleep becomes a habit in childhood or adolescence, it is more likely to continue into adulthood as a part of lifestyle (Cappuccio *et al.*, 2008).

Reduced sleep results from many reasons, including unhealthy lifestyles and sleep disorders (Sun *et al.*, 2009; Wolfson & Carskadon, 1998; Wilson, 2005; Atkinson *et al.*, 2008; Mullington *et al.*, 2009). Sun and colleagues (2009) recently reported in the Toyama Birth Cohort Study that adolescents aged 12-13 y (n = 5753) who spent many

hours watching TV and playing video games tended to delay bedtimes, over-eat, and become physically inactive. In addition to lifestyle choices, the rapid development of shift work, mediating disruption to sleep and circadian rhythms behaviorally and biologically, has contributed to sleep reduction over the past years (Atkinson *et al.*, 2008). Furthermore, sleep disorders and illnesses, such as severe or chronic insomnia, sleep apnea, anxiety and depression, substantially decrease sleep quality and duration (Wilson, 2005).

Sleep loss caused by irregular lifestyle and illness, over the course of weeks, months, and years, has insidious effects on health and quality of life. On the basis of laboratory, population-based and epidemiological studies, sleep-deprived people are more likely to have poor memory (Wilson, 2005), comprehension and attention span (Wilson, 2005), high blood pressure (Kato *et al.*, 2000; Meier-Ewert *et al.*, 2004; Tochikubo *et al.*, 1996), increased inflammation (Bøyum *et al.*, 1996; Dinges *et al.*, 1994), impaired glucose regulation (Gottlieb *et al.*, 2005; Gottlieb *et al.*, 2006; Knutson *et al.*, 2007), type 2 diabetes (Knutson *et al.*, 2007; Ayas *et al.*, 2003; Tuomilehto *et al.*, 2009), obesity (Cappuccio *et al.*, 2008; Knutson *et al.*, 2007; Gangwisch *et al.*, 2005), and cardiovascular diseases (Heslop *et al.*, 2002). Sleep deficit does more than make people prone to unhealthiness; it also boosts the chance of accidents, such as an automobile crash or a near-miss incident partially due to daytime sleepiness (Barger *et al.*, 2005).

2.2 Short Sleep Duration and Obesity in The Context of Energy Balance

2.2.1 The Relationship between Sleep Deprivation and Obesity

A decline in average sleep duration over the past century has paralleled the globally increased obesity prevalence. As investigations showed, the U.S. obese population doubled from 1980 to 2002 (Flegal *et al.*, 2002) and increased to 33.8% in 2008 (Flegal *et al.*, 2010). The statistics for Canada are similar, changing from 6% in 1985 to 16% in 2003 among adults (Katzmarzyk & Mason, 2006). China, one of the leanest populations, is fast in catching up with the West. In fact, the China Health and Nutrition Survey recently reported that the population of overweight adults increased by nearly 40% and that of obesity doubled from 1992 to 2002 (Wu *et al.*, 2009). Alarmingly, owing to etiologic roles of obesity in several chronic diseases such as hypertension, type 2 diabetes and cardiovascular diseases, related health costs have exceeded those resulting from smoking and drinking based on 1998 U.S. national survey data (Sturm, 2002).

The inverse relationship between sleep duration and body mass index (BMI) as well as the susceptibility to overweight or obesity have been demonstrated across all ages and in several ethnic groups. In a cross-sectional study, Gangwisch and colleagues (2005) analyzed longitudinal data from the 1982-1984, 1987, and 1992 Epidemiologic Follow-up Studies of the first National Health and Nutrition Examination Survey and found that adults aged 32-49 y who slept < 7 h/night had higher average BMIs compared to those getting 7 h of sleep. Specifically, subjects with 2-4, 5, and 6 h of sleep had 135%, 60%, and 27% higher frequency of being obese than those sleeping 7 h/night, respectively, after adjusting for potential confounders such as gender, education and ethnicity. Moreover, Cappuccio and colleagues (2008) reported in a meta-analysis (n = 634,511)

that the odd ratios for short sleep and obesity in children (≤ 10 h/night) and adults (≤ 5 h/night) were 1.89 and 1.55, respectively, indicating that inadequate sleep is consistently associated with obesity in different populations. Furthermore, based on the pooled regression analysis, they suggested that a 1 h/d decline in sleep time would contribute to a 0.35 kg/m^2 increase in BMI in adults. Similar findings were reported in case-control and cross-sectional studies of children (Sekine *et al.*, 2002; Bawazeer *et al.*, 2009) and adolescents (Sun *et al.*, 2009; Gupta *et al.*, 2002).

2.2.2 Effects of Sleep Deprivation on Energy Intake

One of the potential mechanisms by which short sleepers have a higher risk of becoming obese is an increase in energy intake, as **Figure 1.1** shows. Many studies focusing on hormonal responses of sleep deprivation have demonstrated that energy intake may be stimulated by disturbing orexigenic/anorexigenic hormones, such as the hunger-suppressing hormone leptin and the appetite-stimulating hormone ghrelin (Cappuccio *et al.*, 2008; Knutson *et al.*, 2007). In a cross-over clinical trial, 12 healthy young men (age, 22 $\pm$ 2 y; BMI, 23.6 $\pm$ 2.0 kg/m^2) were randomly assigned to a 2-d session with 4-h or 10-h sleep (Spiegel *et al.*, 2004). These investigators found that during short sleep subjects had reduced leptin (18%) and elevated ghrelin (28%), accompanied with increased ratings of hunger and appetite by 24% and 23%, respectively, as compared with a period of sleep extension. Moreover, a population-based study involving 1,024 volunteers from the Wisconsin Sleep Cohort Study concluded that sleep duration was inversely related to BMI (Taheri *et al.*, 2004). Subjects with 5-h sleep had leptin levels lowered by 15.5% and ghrelin elevated by 14.9%, compared to subjects with

8-h sleep, independent of BMI. However, fewer studies employed direct food intake measures under sleep interventions. Brondel *et al*. (2010) recently studied the effects of a single night of 4-h short sleep on energy intake in healthy men and found that subjects consumed more energy on the day after sleep restriction, compared with the 8-h sleep duration, whereas Nedeltcheva *et al*. (2009) found that 14-d of 5.5-h sleep had no impact on energy intake in 11 men and women relative to regular sleep duration. Overall, although the influential effects of sleep deprivation on energy intake are robust, more human studies, especially targeting the nonhomeostatic eating behavior associated with short sleep, are still necessary (Chaput *et al.*, 2010).

2.2.3 Effects of Sleep Deprivation on Energy Expenditure

Reduced energy expenditure, as shown in **Figure 1.1**, represents a parallel potential mechanism for the short sleep-obesity association. Total energy expenditure (TEE) generally comprises resting metabolic rate (RMR), thermic effect of food (TEF), and activity energy expenditure (AEE). RMR is the amount of energy produced while digestive system is inactive and the whole body is at rest, accounting for 60-75% of TEE (Mifflin *et al.*, 1990). RMR decreases with age and loss of lean body mass, but increases with muscle mass growth (Denzer & Young, 2003). TEF, normally accounting for 10% of TEE, is generated by digesting and absorbing food (Denzer & Young, 2003). Lastly, energy used for activity accounts for the remaining 15-30% of TEE, and includes both spontaneous and voluntary activities (Mifflin *et al.*, 1990). Specifically, AEE has two major domains: 1) energy expended while undertaking structured and planned physical activities such as sports and workout in gyms; and 2) energy induced by unintentional

activities such as fidgeting. Although sport-like exercise is conventionally encouraged to elevate TEE, this may only account for a small proportion of daily AEE because the duration is limited for a normal person; however, non-exercise activity thermogenesis (NEAT), although individually contributing little to total AEE, tends to be appreciable when accumulated over time because it occurs throughout the day (Dong *et al.*, 2004). Therefore, individuals with different activity levels or personal habits could have significant variance in TEE. Levine *et al*. (2005) in the Mayo Clinic, for instance, has reported that TEE could substantially vary from person to person by as much as 2000 kcal/d. Furthermore, to express several lifestyles with indicative numbers, a term called physical activity level (PAL) was introduced by the Food and Agriculture Organization of the United Nations/World Health Organization/United Nations University (FAO/WHO/UNU) expert consultation (2001), and according to intensity of a person's habitual activity, PAL values were generally classified into three categories: 1) sedentarily or lightly active, 2) moderately active, and 3) vigorously active. PAL can be estimated from TEE and RMR (i.e. PAL = TEE / RMR) to describe a person's activity characteristics (Salbe *et al.*, 1997; Martin *et al.*, 2007). Multiplying PAL by the RMR has been adopted by FAO/WHO/UNU to estimate energy requirements for dietary recommendations since 1985 (Levine *et al.*, 2005; FAO/WHO/UNU, 2001).

Some evidence suggests that a decrease in sleep time may alter the motivation for physical activity behavior. Sleep-deprived subjects tend to reduce exercise or leisure-time activities, either voluntarily or involuntarily, because of excessive daytime fatigue and/or irresistible daytime sleepiness (Briones *et al.*, 1996; Mackintosh, 2001; Weaver *et al.*, 1997). Moreover, night-time workers usually feel exhausted and have to sleep during the

day, which conflicts with regular daytime activities such as team sports, group activities or organized events (Atkinson *et al.*, 2008). Physiologically, some hormones, such as leptin and ghrelin, were found associated with alterations in energy expenditure particularly in animal models (Scarpace & Matheny, 1997; Tang-Christensen *et al.*, 2004). However, hormonal effects of sleep reduction on energy expenditure in humans are still unclear (Hukshorn *et al.*, 2000; Hukshorn *et al.*, 2003; Mackintosh & Hirsch, 2001; Westerterp-Plantenga *et al.*, 2001). Converse to the decreasing effect of sleep deprivation on energy expenditure, the early concept proposed by Zepelin and colleagues (1974) indicates that daily exposure to extra hours of wakefulness may be accompanied with elevated out-of-bed activity. A recent clinical trial by Brondel *et al.* (2010) was in line with this concept, showing an increase in physical activity after the short sleep session, and some epidemiologic studies also reported similar findings (Patel *et al.*, 2008; Chaput *et al.*, 2008). Due to the existence of these conflicting results concerning physical activity, the relationship between sleep deprivation and energy expenditure in humans constitutes a matter of debate. One of the main contributors to this controversy may be variable measurement methods with different degrees of accuracy and precision used across studies. Therefore, understanding the advantages and disadvantages of available methods is particularly important in designing a study using energy expenditure measurements.

2.3 Measurement Methods for Energy Expenditure

2.3.1 Direct versus Indirect Calorimetry

Direct calorimetry measures total heat dissipated by evaporation, radiation, conduction and convection from the body (Jequier, 1985). While this technique is able to

measure TEE accurately, the subject has to be placed in a thermally-isolated chamber which restricts the person's activity to some extent. Therefore, direct calorimetry does not reflect true free-living TEE. Indirect calorimetry, which is used more often, assesses energy expenditure by measuring oxygen consumption (VO_2), but subjects have to wear a mouthpiece, hood or reside in a whole body chamber, interfering substantially with activities of daily living (Jequier, 1985; Jequier, 1996). Additionally, only one individual can be monitored at a time for both direct and indirect calorimetry, making these techniques very time consuming if applied to large populations (Jequier, 1985; Lagerros & Lagiou, 2007).

2.3.2 Activity Monitors - Heart Rate Monitors, Accelerometers, and Pedometers

Along with fast technological advances, more and more electromechanical activity monitors including heart rate monitors, accelerometers, and pedometers have been developed and proliferated with great improvement during the past decades (Trost, 2001). Having advantage of low cost, portability, a function of information storage, and easiness in implementation, activity monitors are commonly used in research as well as our daily lives for assessing activity or energy expenditure (Trost, 2001; Bassett Jr., 2000; Dollman *et al.*, 2009; Reilly *et al.*, 2008). Heart rate monitors, for instance, assess physical activity based on the linear relationship between heart rate and VO_2. These monitors are typically strapped around the chest, sometimes presenting burdens to subjects (Trost, 2001). Many validation studies have been conducted, showing a reasonably high agreement between energy expenditure estimated by heart rate monitors

and doubly labeled water methods (Eston *et al.*, 1998; Ballor *et al.*, 1989; Livingstone *et al.*, 1992; Kohl III *et al.*, 2000b). However, some studies indicated that heart rate monitors tended to have delayed response to sudden changes in movement. This lapse may mask sporadic and intermittent patterns of activity, particularly common with children and adolescents (Trost, 2001; Dollman *et al.*, 2009; Eston *et al.*, 1998). In addition, the accuracy of heart rate monitors is poor for low-intensity activity assessments (Bassett Jr. *et al.*, 2000) and may be influenced by several factors including emotional stress, body temperature, fitness, and medication (Dollman *et al.*, 2009; Lamonte & Ainsworth, 2001).

The accelerometer is another tool used to assess frequency, intensity, and duration of movements by detection of acceleration and deceleration (Ainslie *et al.*, 2003). Accelerometers can be worn on arms, wrists, the waist, thighs, legs, or ankles. Using multiple sites is frequently adopted to improve accuracy of assessing whole-body movements (Foerster & Fahrenberg, 2000). Substantial evidence suggests that accelerometer-generated energy expenditure is moderately or highly correlated with those measured by validation standards, such as indirect calorimetry and the doubly labeled water (DLW) method (Kohl III *et al.*, 2000b; Noland *et al.*, 1990; Bray *et al.*, 1994). Moreover, the newer triaxial accelerometer has even greater accuracy than older uniaxial models (Bouten *et al.*, 1994; Yamada *et al.*, 2009). However, detectability, to some extent, depends on activity characteristics. For example, Terrier *et al.* (2001) observed that accelerometers usually underestimate/overestimate energy expenditure for activities like uphill/downhill walking which involve slope variation of bodies while moving. Basically, accelerometers were found very poor at detecting static activities, such as standing and

brain work, upper body movements, vertical lift or swimming; thus, the gross energy expenditure of a person who conducts considerable arm work with moderate to vigorous intensity, for example, is probably underestimated by accelerometers (Bassett Jr. *et al.*, 2000; Hendelman *et al.*, 2000; Welk *et al.*, 2000; Washburn *et al.*, 1993). Despite these weaknesses, accelerometers usually provide better assessments of overall levels of physical activity in comparison with heart rate monitors (Trost, 2001; Eston *et al.*, 1998; Welk *et al.*, 1998). Studies conducted by Eston *et al*. (1998) concluded that the correlation between oxygen consumption and Tritrac accelerometer ($r = 0.91$) was notably higher than that of observed for heart rate monitors ($r = 0.8$). Moreover, Welk *et al*. (1998) concluded that heart rate monitors only provided valid assessments at moderate- to high-intensity activity, but accelerometers, in contrast, provided valid activity assessments with low to high intensity. To improve accuracy and precision of energy expenditure assessments, several investigators have tried a combination of these two techniques, demonstrating that the errors were the lowest with the combined technique to predict free-living energy expenditure (Treuth *et al.*, 1998). It is worth noting that these approaches are only suitable for small to medium sized population studies because of their relatively high cost ($150-500 per unit) (Trost, 2001; Shephard, 2003).

Electronic pedometers, often referred to as step counters, are newly developed, inexpensive, easy to use, devices that have potential to provide reliable measures of ambulatory activity. Pedometers can provide derived outcomes, depending on the brand, which include travelled distance, calories and time spent at specific time period (Tudor-Locke *et al.*, 2009). The device is usually arm-, waist-, thigh-, leg-, or ankle-mounted,

giving little burden to the subject. Pedometers have a number of useful applications in which they are often used in physical activity promotion campaigns to help motivate participants to exercise (Lindberg, 2000); they are also used in clinical studies which employ walking interventions (Croteau, 2004; Swartz *et al.*, 2003); moreover, they have been used to describe activity levels of walking-related activities in epidemiological studies worldwide (McCormack *et al.*, 2003) and compare the patterns among different populations across gender, race or countries (Vincent *et al.*, 2003). Previous studies focusing on the validity of electronic pedometers have found high correlations between steps recorded by pedometers and scaled VO2 or direct observation during walking, running and unstructured playing activities in children (Eston *et al.*, 1998; Kilanowski *et al.*, 1999). Furthermore, Bassett Jr. *et al*. (1996) concluded that the newer, commercially available pedometer, Yamax DW-500, could estimate walking distance and the number of steps with reasonable accuracy. In addition, pedometers which cost < $100 per unit have an advantage of becoming a cost-effective alternative to accelerometers ($150-500 per unit) to measure physical activity, especially for small budget feasibility studies and large-scale epidemiological and surveillance studies (Trost, 2001). Yet, similar to accelerometers, pedometers are insensitive to non-locomotive forms of activity, such as cycling and stair climbing. With the complex nature of daily activity that people can engage, pedometers are considered less accurate for assessing energy cost (Crouter *et al.*, 2003). As Sequeira *et al*. (1995) demonstrated, pedometers are unable to detect the intensity of static activities, such as lifting and pushing, so energy spent on these kinds of heavy work is usually underestimated. Likewise, many studies have noted that the accuracy of pedometers in step counting is influenced by speed of movement (Crouter *et*

al., 2003; Beets *et al.*, 2005; Mitre *et al.*, 2009; Nakae *et al.*, 2008; Duncan *et al.*, 2007). Pedometers tend to undercount steps at low speed, but the accuracy improved with increasing speed. In addition to this limitation, pedometers are unable to provide information about frequency, intensity or duration of physical activity (Trost, 2001).

2.3.3 Activity Questionnaires

Compared to other techniques, activity questionnaires (AQs) are the easiest to distribute and administer, and do not require much motivation or time from subjects (Lagerros & Lagiou, 2007; Ainslie *et al.*, 2003). AQs provide considerable information on physical activity as well as other factors in large numbers of subjects, exerting a clear advantage for large population-based or epidemiological studies. The standard format of an AQ consists of diverse activities categorized according to intensity, and corresponding frequency and duration (Lagerros & Lagiou, 2007). AEE can be calculated by multiplying reported hours by the corresponding metabolic equivalent value (MET) assigned to various activities, and then expressed on a per day basis.

Nowadays, validity issues of AQs have been raised since significant discrepancies exist between AQ-derived AEE and AEE derived by other techniques (Carrasco & Jover, 2003). Validation and comparison studies have explored the reliability, validity and sensitivity of published AQs, and potential limitations have been identified (Lagerros & Lagiou, 2007; Ainslie *et al.*, 2003). The review from Neilson *et al*. (2008), for instance, found that only 2 out of $\geq$ 20 AQs, covering both sexes, all ages, obese and non-obese people, and international measurements, had acceptable criterion validity. The majority of tested AQs were unreliable due to many factors such as disregarding of non-exercise

activities. Likewise, AQs are highly susceptible to subject bias and mis-reporting, typically over-reporting (Lagerros & Lagiou, 2007). By and large, limitations of AQs indeed preclude us from drawing firm and reliable conclusions from research which involves EE measures as influential parameters. Nevertheless, as pointed out by Sesso (2007), self-reported methods of data collection may remain the primary way to quantify physical activity in epidemiological or large-scale studies because they are cost-effective and can collect large amount of information for research.

2.3.4 Doubly Labeled Water Method

In 1955, the DLW method was developed by Lifson *et al*. (1955), and its first application in humans for free-living TEE measurements was published in 1982 (Schoeller & Van Santen, 1982). Water contains two rare heavy isotopes, ^{2}H, also called deuterium, and ^{18}O, both of which are safe because the typical dose given falls well below levels that cause side-effects, damage or toxicity (Jones, 1990). To date, the DLW method has been utilized in a variety of groups, including premature infants, children, adolescents, adults, pregnant and lactating women, the elderly and hospitalized patients (FAO/WHO/UNU, 2001; Ainslie *et al.*, 2003; Schoeller & Van Santen, 1982; Jones, 1990; Schoeller, 1999; Schoeller, 1988; Wolfe, R.R., Chinkes, D.L., 2005). Applications of the method include assessing energy requirements, validating other available methods used to measure energy intake or physical activity, and understanding the effects of dietary and/or physical activity interventions on health (Ainslie *et al.*, 2003).

The fundamental basis of the DLW method, as shown in **Figure 2.1**, is that ^{18}O component, after mixing with body water, is eliminated as CO_2 and H_2O, whereas ^{2}H is

excreted solely as H_2O (Wolfe & Chinkes, 2005); hence, ^{18}O turnover is quicker than ^{2}H. Sequentially, the difference between elimination rates provides a measure of CO_2 output, using valid prediction equation (Speakman, 1998). Based on the same physiological principle as indirect calorimetry, CO_2 production combined with standard equations can be used to predict TEE (Schoeller, 1999). However, different from calorimetry, the DLW method allows subjects to freely perform normal activities of daily living because the method relies on collections of urine and saliva samples rather than heat or respiratory gases collected by a thermally-isolated chamber or a ventilated hood, respectively (Jequier, 1985). Briefly introducing the laboratory procedures, a specific dose of DLW is given to an individual according to weight after the collection of baseline urine and saliva samples. After 3 h and 4 h, post-dose saliva samples are collected separately for future determination of total body water (TBW); subsequently, urine samples are collected at different time points for determination of isotopic elimination rates within this time period (Schoeller, 1999). Based on previous investigations, the biological half-lives of stable isotopes in DLW averaged between 8 d and 10 d for healthy humans (Schoeller & Van Santen, 1982; Schoeller & Webb, 1984; Schoeller *et al.*, 1986). Therefore, the length of study periods usually varies from 4 to 21 d depending on activity levels of study groups (Ainslie *et al.*, 2003). Athletes, for example, usually eliminate body water and produce CO_2 faster than sedentary individuals because of their vigorous activity levels, so the study period can be as short as 4 d in order to avoid complete elimination of administered isotopes. In general, to avoid imprecise results related to analytic errors, study periods should be long enough for sufficient elimination of isotopes but short

enough so that isotope enrichments in the final urine samples are detectable (Schoeller, 1988).

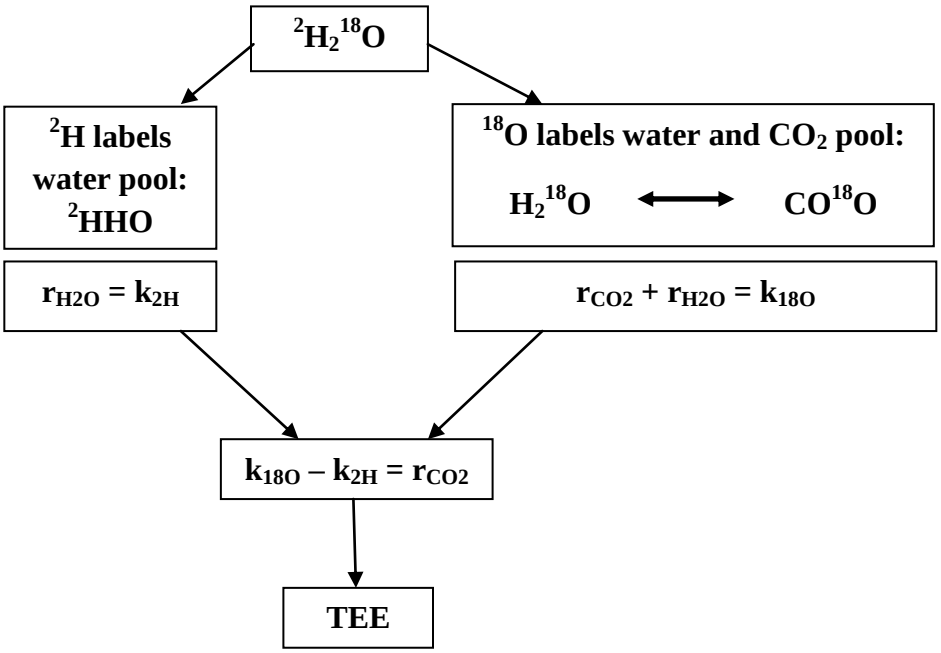

Figure 2.1 The theory of the doubly labeled water method

$^{2}H^{18}O$, doubly labeled water with ^{2}H and ^{18}O labeled isotopes.

r_{H2O} and r_{CO2}, elimination rates of H_2O and CO_2, respectively.

k_{2H} and $k_{18O,}$ turnovers of ^{2}H and ^{18}O isotopes, respectively.

r_{CO2}, CO_2 production rate.

TEE, total energy expenditure.

Several assumptions have been made in the DLW method to ensure its validity **(Table 2.1)**: 1) the volume of water pool for labeled isotope distributions is constant; 2) flux rates of water and CO_2 are constant; 3) ^{2}H is washed out only as water whereas ^{18}O is lost as water and CO_2; 4) enrichments of water and CO_2 exiting the body are the same as those remaining in body water; and 5) no isotopic exchange occurs between the body system and environmental water / CO_2 through skin or lungs (Schoeller, 1988). Consequently, TEE estimates may be inaccurate if DLW is applied to persons with disorders, such as kidney and respiratory disorders, which may alter TBW pool or fluxes of water and CO_2. Any environmental factors that influence ambient temperatures, water turnover rates (Murgatroyd *et al.*, 1993), or background isotope levels will also affect accuracy (Horvitz & Schoeller, 2001). Also, random error and analytical variance still exist during the assessment and the confidence intervals for individual TEE measured by DLW sometimes could be relatively large (Schoeller & Hnilicka, 1996). Thus, this method perhaps may not be able to resolve small differences between groups.

Table 2.1 The basic assumptions of the doubly labeled water method (Schoeller, 1988)

Assumptions	Imperfections
1. The volume of the water pool in which the isotopes are diluted is constant	- Episodic eating and drinking behaviors - Weight gain/loss - Infant growth
2. The fluxes of water and CO_2 are constant	- Water intake and physical activity are episodic
3. Isotopes are distributed only in body water, labeling body water and CO_2 only	- Each isotope exchanges to small degree with nonaqueous molecules - Hydrogen dilution space/1.04 = oxygen dilution space/1.01 = total body water - Deviations from a smooth exponential isotopic elimination curve
4. Enrichments of water and CO_2 exiting the body are the same as those in body water	- Isotope fractionation factors for ^{2}H and ^{18}O, eg. breath water, non-sweat transcutaneous water and CO_2
5. No water or CO_2 enters the body via skin or lungs	- Exchange with environmental water and CO_2 has been demonstrated, but the error is usually quantitatively unimportant to measuring CO_2 production because the elimination rates of hydrogen and oxygen are affected equally

In general, the DLW method is able to accurately estimate free-living TEE of individuals with varying lifestyles or even those with atypical levels of activity (Speakman, 1998). Its noninvasive nature causes little interference with human behavior, so subjects can freely participate in daily activities throughout the study period (Ebine *et al.*, 2000). Unfortunately, the relatively high cost of DLW purchase and high demand for specialized expertise for mass spectrometry instrumentation limit its widespread utilization in moderate- to large-scale studies.

Taking an overall look at these techniques for energy expenditure assessments introduced above, they enhance the ability to effectively measure physical activity along with technical advances. However, no single measurement tool works perfectly across all studies. Therefore, understanding their strengths and limitations before embarking on a study or project evaluation is crucial for the success of research into physical activity (Dollman *et al.*, 2009). Measures from calorimetry and the DLW method are relatively precise and accurate, but are not appropriate for larger population-based studies due to limitations on cost and feasibility; comparatively, activity monitors and questionnaires show relatively lower validity because they cannot capture all types of activity, but are often applied to moderate- to large-scale studies (Kohl III *et al.*, 2000b). It has been suggested that using a combination of multiple tools may enhance correlations with validation standards for energy expenditure assessments (Trost, 2001; Eston *et al.*, 1998; Treuth *et al.*, 1998).

Chapter 3: Objectives and Null Hypotheses

Despite the existing evidence of the importance of sufficient sleep (7-8 h/night for adults) for good health, it has been estimated that North Americans sleep only 6.5 h/night or less (Charlotte *et al.*, 2008; Fok *et al.*, 2007) and the sleep time is still dropping (Charlotte *et al.*, 2008). Sleep deprivation, based on a large number of laboratory, population-based and epidemiological studies, has been found to associate with increased risk of weight gain and obesity across genders, age and ethnic groups (Sun *et al.*, 2009; Cappuccio *et al.*, 2008; Gangwisch *et al.*, 2005; Sekine *et al.*, 2002; Bawazeer *et al.*, 2009; Gupta *et al.*, 2002). It has been shown that insufficient sleep may disturb the energy intake side of the energy balance equation via increased appetite and hunger (Cappuccio *et al.*, 2008; Knutson *et al.*, 2007). With regard to the impact of reduced sleep on energy expenditure, no firm conclusions have been drawn due to the heterogeneity of results amongst studies (Marzullo *et al.*, 2004; Van Cauter *et al.*, 2007). Therefore, the present clinical trial was proposed to investigate the metabolic consequences of short-term sleep restriction in healthy young adults in terms of energy intake and energy expenditure with following objectives and null hypotheses:

Objective 1: To determine whether sleep duration affects energy or food intake in healthy young adults.

Null hypothesis 1: There will be no difference in energy or food intake between short sleep and habitual sleep.

Objective 2: To investigate the impact of sleep duration on free-living energy expenditure as well as physical activity levels in healthy young adults using the doubly labeled water method.

Null hypothesis 2: Energy expenditure and physical activity levels estimated during a period of short sleep duration will not be different from those measured during a period of habitual sleep for the same group of subjects.

Objective 3: To find out the overall impact of sleep duration on energy balance.

Null hypothesis 3: There will be no detectable difference in energy balance as a function of sleep duration.

Chapter 4: Materials and Methods

4.1 Study Participants

Subjects were recruited in New York City through internet advertisements. Inclusion criteria were 30-45 y of age and BMI between 22 and 25 kg/m^2. This age range was selected because the significant association between sleep duration and obesity was found in the 32-49 age group, according to Gangwisch *et al*. (2005); and the BMI range was decided because of the potential impact of sleep duration on energy balance and future weight changes. Overweight and obese individuals usually have positive energy balance and may have already experienced a complex of behavioral and physiological effects of short sleep. Additionally, all subjects had habitual sleep of 7-9 h/night without the daytime nap habit to ensure that the degree of sleep restriction was similar across individuals.

Habitual sleep time was verified in the 2-wk screening period prior to the first study phase using actigraphy (Actiwatch-Mini-Mitter Co, Inc., Bend, OR) and sleep diaries. Health conditions and other eligibility criteria were also checked. It was required that average sleep time over the 14 nights of screening fell within 7-9 h range, with at least 10 nights with $\geq$ 7 h of sleep and no more than 4 nights with $\leq$ 6 h of sleep. Individuals were excluded if they had any sleep, psychiatric, or eating disorders (e.g. sleep apnea, involuntary sleep movement), depression, or type 2 diabetes. Smokers, shift workers, drowsy drivers, drug and alcohol abusers, those with excessive caffeine intake (> 300 mg/d), pregnant women, persons who traveled across time zones during the past 4 weeks or whose work required long-distance driving or operating heavy equipment, or individuals with recent weight fluctuation were excluded. Finally, 30 eligible participants

(15 males and 15 females) were enrolled in the study and randomly assigned to a sleep sequence.

4.2 Study Protocol

The study protocol **(Figure 4.1)** was approved by the Institutional Review Boards of St. Luke's/Roosevelt Hospital Centre and Columbia University (New York, NY). The study followed a randomized crossover design on 2 separate test conditions: short (4 h/night) and habitual (9 h/night) sleep. Daily sleep duration was verified and quantified using the Sandman Sleep Data Recording System SD-64 (Nellcor Puritan Bennett Ltd, Kanata, Ontario, Canada) with Sandman Elite Sleep Diagnostic Software version 8.0. Each phase was conducted for 6 consecutive d with 5 nights, which has been shown to effectively trigger endocrine and metabolic alterations (Knutson *et al.*, 2007; Spiegel *et al.*, 2004). A 4-wk washout period between two phases was shown sufficient to recover (Kohl III *et al.*, 2000a) from the previous sleep phase. During both inpatient phases, subjects stayed at Clinilabs, a sleep research laboratory (New York, NY) which has private bedrooms as well as other work, play and exercise facilities. Therefore, participants lived freely at Clinilabs, engaging in leisurely activities or home-office-type work and having free access to internet, telephones, televisions, videos, reading, and a gym. Every morning, participants were weighed first after getting up and then served with breakfast. Every night, volunteers were required to go to bed at either 10 p.m. or 1 a.m. and wake up at either 7 a.m. or 5 a.m. the following morning for the 9-h or 4-h sleep conditions, respectively. No other sleep or naps were permitted during 2 phases. Sleep technicians played an important role in this study. They had to prevent subjects from

falling asleep during the day and keep them awake until 1 a.m. during the sleep restriction phase.

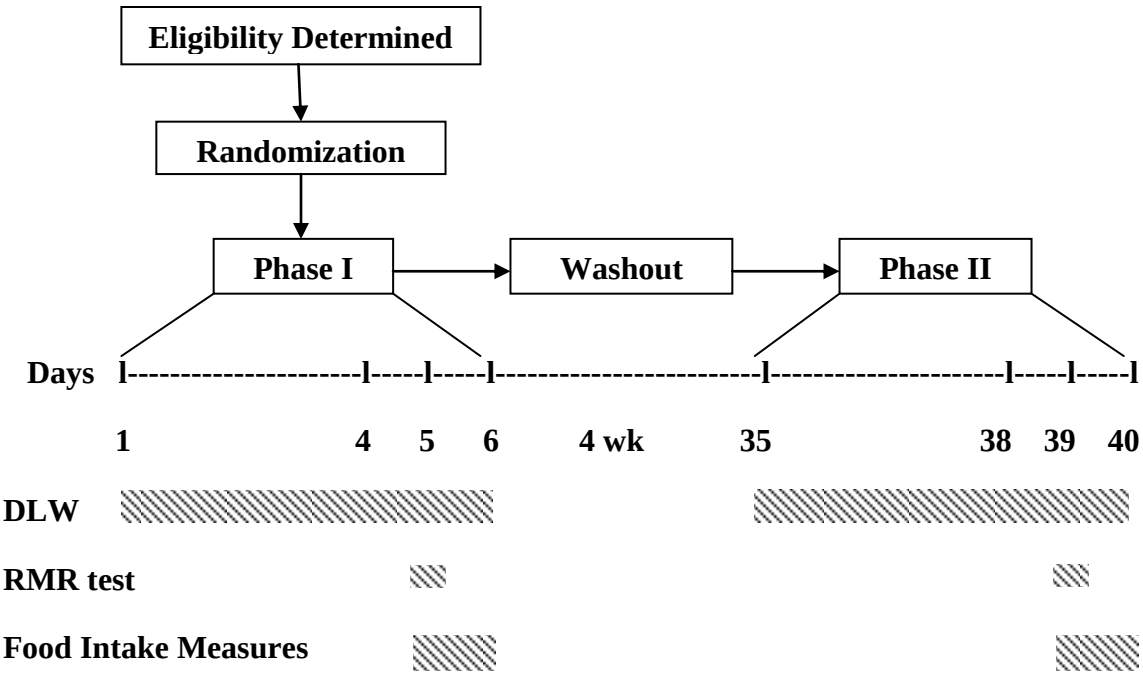

Figure 4.1 Overview of the study protocol

DLW, doubly labeled water method used to measure total energy expenditure.

RMR, resting metabolic rate measured by indirect calorimetry.

Food intake was measured by weighing foods before and after eating.

During the first 4 d of each phase, subjects were given a controlled, weight maintenance diet meeting their energy requirements estimated from Harris-Benedict (1918) equation. Three customized isocaloric meals, providing 90% of total energy requirements (TERs), were nutrient-rich and palatable, and served at 8 a.m., 12 p.m., and 7 p.m. One mid-afternoon snack containing 10% of TER was served at 4 p.m. On d 5 and

d 6, subjects self-selected their foods and non-alcoholic beverages with nutrition information available. Ad libitum food intake was measured by weighing before and after each meal. Eating occasions that were ≥ 20 min apart were recorded by study personnel as well. Energy and macronutrient intakes were analyzed using Diet Analysis Plus Software version 8.0 (Wadsworth, Florence, KY). However, subjects were discharged at 8 p.m. on d 6, ending up with incomplete dietary record for d 6, so food intake assessment was only based on d 5 data.

On the morning of d 5, RMR measurements took place at the Body Composition Unit of St. Luke's/Roosevelt Hospital. Briefly, subjects were instructed to stay fasted (≥ 12 h) and physically inactive (≥ 30 min) before the test. During the measurement, they were awake and lay quietly and motionless. RMR was then measured using a plastic ventilated hood indirect calorimetry system (Delta-Trac II metabolic monitor; SensorMedics, Yorba Linda, CA) over a 40- to 60-min interval (Jones Jr. *et al.*, 2004). VO_2 and carbon dioxide production (VCO_2) were recorded and analyzed, and then gas exchange rates were used to calculate RMR (Jequier, 1985; Weir, 1949). RMR which accounts for 60-75% of TEE (Mifflin *et al.*, 1990) was measured first to access whether it differed individually between habitual and short sleep durations; secondly, it was used in the calculation of PAL and AEE after knowing TEE values. Respiratory quotient (RQ) was calculated simultaneously using VO_2 and VCO_2 values.

4.3 Assessments of Energy Expenditure

4.3.1 Doubly Labeled Water Method Protocol

The DLW method, as the gold standard for free-living energy expenditure assessment, was chosen to estimate individual energy expenditure during both short and habitual sleep conditions. The DLW protocol was conducted over a 6-d period at the termination of each phase. On d 1 morning after subjects arrived at Clinilabs, a urine sample and a saliva sample were collected after overnight fasting to determine baseline isotope enrichments. Then, subjects were given a single oral dose of DLW consisting of 0.10 g 10 atom percent excess (APE) of ^{18}O and 0.08 g 99.8 APE of ^{2}H per kg body weight followed by a water rinse. Subsequently, post-dose saliva was sampled at 3 h and 4 h time points for determination of TBW from ^{2}H isotope dilution space. On the mornings of d 2 and d 6, enriched urine samples were collected separately to determine elimination rates for the two isotopes (^{2}H and ^{18}O). When subjects returned to Clinilab after the washout period, the same DLW protocol was run for their second phase. All samples were stored in a -20°C freezer until the shipment to the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) for isotopic analysis.

4.3.2 Laboratory Preparations and Analyses

Preparations of samples were conducted off-line. Urine samples were first mixed with carbon charcoal which effectively absorbed impurities and separated from fluid; then, the mixture was filtered through a 0.45 μm nylon membrane using a 25 mm syringe filter; and finally, the purified urine was placed into a 2 ml autosampler vial. The saliva sample preparation was relatively simpler which only involved the usage of a centrifuge

to precipitate impurity, and then the upper layer of fluid was placed into a 2 ml autosampler vial. Isotopic enrichment was measured using an automated high temperature conversion elemental analyzer (Thermo Finnigan TC/EA, Germany) in combination with an isotope ratio mass spectrometer (IRMS: Thermo Finnigan DELTA^{plus} V, Germany). Briefly, an autosampler first injected 0.5 µl of each sample into a glassy carbon reactor sealed in the TC/EA kept at 1450 °C. All hydrogen and oxygen in the fluid sample was then converted to H_2 and CO, respectively, during pyrolysis. Subsequently, the gaseous products were separated by a 5 Å packed gas chromatographic column (90 °C) and then were transferred to the IRMS for detection. All measurements were carried out with continuous helium flow (> 90 ml/min) to prevent contamination with traces of water, oxygen and nitrogen. The ratios of $^{2}H/^{1}H$ and $^{18}O/^{16}O$ were analyzed by IRMS against Vienna Standard Mean Ocean Water (V-SMOW). Within a 48-h period of analysis, the IRMS was calibrated using V-SMOW, Greenland Ice Sheet Precipitation (GISP), 302A (moderately enriched water), and IA-R056 (highly enriched water) standards. All of these standard waters were purchased from International Atomic Energy Agency (IAEA, Vienna, Austria). Regression analysis of these standards established good linearity of response from low to high enrichments. Each sample was measured minimal five times depending on the extent of memory effects which were residual effects from the previous sample causing the first one or two enrichment values of following measured samples to be unreliable. Therefore, only the last values with small variance were picked for mean calculations.

4.3.3 Energy Expenditure Calculations and Analyses

TBW was calculated based on ^{2}H dilution in healthy individuals (Schoeller *et al.*, 1980). ^{2}H dilution space (DS_d) in kg was calculated relying on ^{2}H enrichment measures of saliva samples at baseline and 3 and 4 h post-dose as below,

$$DS_d = \frac{d}{MW} \times \frac{APE}{100} \times 18.02 \times \frac{1}{R_{std} \times \Delta\delta_{2_H}}, kg \quad \text{I}$$

Where d (g) is the dose of $^{2}H_2O$, MW is the molecular weight of $^{2}H_2O$, APE is atom percent excess of ^{2}H, R_{std} is the ratio of $^{2}H/H$ in the standard (1.5576×10^{-4}), and $\Delta\delta_{2_H}$ is the enrichment change between pre- and post-dose saliva samples in per mil units (‰). Subsequently, TBW (kg) was calculated as $DS_d/1.041$ (Racette *et al.*, 1994).

According to the theory of the DLW method, the difference in isotopic turnovers is crucial to CO_2 production. Using the two-point approach, elimination rates of ^{2}H (k_h) and ^{18}O (k_o) were computed from dividing changes in enrichment ($E_f - E_i$) by the corresponding time difference ($t_f - t_i$). Subsequently, CO_2 production rates were calculated using the formula (Ebine *et al.*, 2000)

$$rCO_2 = 0.4554 \times TBW \times (1.007 \times k_o - 1.041 \times k_h) \quad \text{II}$$

where rCO_2 is CO_2 production rate in mol/d, TBW is total body water volume in mol, and k_o and k_h (d^{-1}) are elimination rates of ^{18}O and ^{2}H, respectively.

Finally, TEE (kcal/d) were determined from rCO_2 and food quotient (FQ) assumed as 0.85 in the present study (Schoeller *et al.*, 1986) using a modified version of the Weir formula (Weir, 1949)

$$TEE = 22.4 \times \left(3.9 \times \frac{rCO_2}{FQ} + 1.1 \times rCO_2\right), kcal/d \quad \text{III}$$

Having TEE and RMR values estimated by the DLW method and indirect calorimetry,

respectively, individual PAL values were calculated simply using the formula below (Nedeltcheva *et al.*, 2009; FAO/WHO/UNU, 2001; Saris, 1998),

$$\mathrm{PAL} = \frac{\mathrm{TEE}}{\mathrm{RMR}} \qquad \text{IV}$$

As mentioned previously, TEE contains the thermic effect of food (usually accounting for 10% of TEE), RMR and AEE. Therefore, AEE was determined as such,

$$\mathrm{AEE} = 0.9 \times \mathrm{TEE} - \mathrm{RMR}, \text{ kcal/d} \qquad \text{V}$$

4.4 Assessments of Energy Balance

Because two types of feeding, controlled for the first 4 d and ad libitum on d 5 and d 6, were employed in the 2 sleep conditions, energy balance was considered according to the study time points. The first time interval was from d 1 to d 4, when controlled diets were given based on individually estimated energy requirement (EER) and energy balance was calculated as such,

$$\text{Energy balance (d 1 – d 4)} = \mathrm{EER} - \mathrm{TEE}, \text{ kcal/d} \qquad \text{VI}$$

The second time interval was d 5 when ad libitum energy intake was completely recorded and measured. The corresponding energy balance was calculated by subtracting TEE from energy intake only on d 5.

Overall daily energy balance during a period of short or habitual sleep phase was also considered. However, the average energy intake per day was calculated differently from the previous two as below,

$$\text{Energy intake (d 1 – d 5)} = (\mathrm{EER} \times 4 + \text{weighed intakes on d 5}) / 5, \text{ kcal/d} \qquad \text{VII}$$

Given TEE (kcal/d) during a period of short or habitual sleep, we then calculated overall energy balance as below,

Energy balance (d 1 – d 5) = Energy intake (d 1 – d 5) – TEE, kcal/d VIII

4.5 Statistical Analysis

4.5.1 Sample Size and Power

This study performed a sample size calculation using the basic formula as (Chow *et al.* 2002):

$$n = (Z_{1-\alpha/2} + Z_{1-\beta})^2 / \delta^2$$

where $Z_{1-\alpha/2}$ is the typical value for the significance level adjusted for multiple comparisons within one hypothesis, α is the desired 2-tailed type 1 error rate, $(1-\beta)$ is the power and δ is the effect size of 0.74. We required 24 samples for 2-sided test to obtain 80% power of rejecting the null hypothesis of no significant difference observed in each pair-wise comparison. The significance levels was $\alpha=0.05$. Allowing for an estimated 20% drop-out rate, we therefore recruited 30 subjects initially. Equal numbers of men and women were included to guarantee the maximum power to examine sex differences in all hypotheses. With 30 subjects, using a crossover design, we should have 80% power to detect differences in RMR of 45 kcal/d, which transfer into TEE differences of 75 kcal/d.

4.5.2 Data Analysis

The study initially enrolled 30 participants (15 men and 15 women), and 27 (14 men and 13 women) completed. Data from 26 subjects (13 men and 13 women) were used for energy and food intake analyses because one man was an outlier. He ate more

than twice as much in long sleep phase as in short sleep which was much more than the average during that phase. Data of 26 subjects (14 men and 12 women) were included in the RMR analysis because one woman who exercised in the morning before the RMR measurement in the short sleep phase was excluded. However, the man excluded from energy and food intake analyses remained in the RMR analysis because this measurement was taken before free-eating started. A total of 26 participant sample sets (14 men and 12 women) were shipped to RCFFN for isotope measurements and energy expenditure determinations. However, one sample set was excluded because all enrichment values of his baseline urine sample were as high as his enriched samples, which was unrealistic. After obtaining individual TEE and RMR, PAL values and AEE were calculated for individuals. Combining energy input and output data, energy balance (n = 24) was obtained by subtracting TEE from energy intake was analyzed as well.

A simple unpaired t-test was employed to compare the difference in sleep time during the same phase across gender. Pairwise comparisons of body weight, energy and food intakes, eating occasions, RMR, TEE, AEE, PAL values, and energy balance between 2 sleep conditions were performed using the paired t-test. Tests were done for combined gender and across gender. Data were expressed as mean $\pm$ SEM and a p value ≤ 0.05 was considered significant. Statistical Analysis Software version 9.2 (SAS 9.2) was applied for data analysis.

Chapter 5: Results

5.1 Participant Characteristics and Sleep Duration

Anthropometric characteristics and background information of participants (14 men and 13 women) are summarized in **Table 5.1**. The mean age of participants was 35.3 $\pm$ 1.0 y (men: 36.6 $\pm$ 1.5 y, women: 33.9 $\pm$ 1.2 y) and BMI was 23.6 $\pm$ 0.2 kg/m^2 (men: 24.1 $\pm$ 0.3 kg/m^2, women: 23.0 $\pm$ 0.3 kg/m^2). Of the 27 participants, 13 were white, 5 were black, 6 were Hispanic, and 3 were others. Participants had different educational backgrounds, including college (n = 3), college graduates (n = 14) and university graduates (n = 9). At the time of study enrollment, they were full-time (n = 1) or part-time (n = 12) employees, self-employed (n = 4), or unemployed (n = 9). EERs were calculated based on age, weight and height of individuals (Harris & Benedict, 1918) and employed in calorie calculations for the controlled diets given during the first 4 d of both phases. The average calories provided to men and women were 2310 $\pm$ 52 and 1805 $\pm$ 36 kcal/d, respectively, to maintain weight and minimize effects of food intake on hormones.

Table 5.1 Participant characteristics

Characteristics	All (n = 27)	Men (n = 14)	Women (n = 13)
Age, y	35.3 ± 1.0	36.6 ± 1.5	33.9 ± 1.2
Height, cm	172.0 ± 1.8	178.6 ± 1.7	164.9 ± 1.8
Weight, kg	70.2 ± 2.0	77.3 ± 2.3	62.5 ± 1.5
BMI, kg/m^2	23.6 ± 0.2	24.1 ± 0.3	23.0 ± 0.3
Estimated energy requirement, kcal/d	2067 ± 59	2310 ± 52	1805 ± 36
Race	White = 13	White = 8	White = 5
	Black = 5	Black = 3	Black = 2
	Hispanic = 6	Hispanic = 2	Hispanic = 4
	Asian/Pacific Islander = 1	Asian/Pacific Islander = 0	Asian/Pacific Islander = 1
	Other/Mixed = 2	Other/Mixed = 1	Other/Mixed = 1
Education	Some college = 3	Some college = 1	Some college = 2
	College graduate = 14	College graduate = 8	College graduate = 6
	Graduate degree = 9	Graduate degree = 5	Graduate degree = 4
Employment	Full-time = 1	Full-time = 1	Full-time = 0
	Part-time = 12	Part-time = 6	Part-time = 6
	Self-employed = 4	Self-employed = 1	Self-employed = 3
	Student = 1	Student = 1	Student = 0
	Unemployed = 8	Unemployed = 5	Unemployed = 3

All values are mean ± SEM.

BMI, body mass index.

Nightly sleep duration during the habitual and short sleep phases are shown in **Table 5.2**, which were all within the desired ranges. On average, participants slept 7.6 ± 0.1 h (approximately 456 min) during habitual sleep compared to 3.8 ± 0.0 h (approximately 228 min) during short sleep. Men slept 7.4 ± 0.1 h during habitual sleep, which was less than women (7.8 ± 0.1 h, $p = 0.01$). When sleep was restricted, men and women only slept 3.8 ± 0.0 h and 3.8 ± 0.0 h, respectively.

Table 5.2 Average sleep duration during each study period

Phases	Parameters, h	All (n = 27)	Men (n = 14)	Women (n = 13)
Habitual sleep	Time in bed	9.0 ± 0.0	9.0 ± 0.0	9.0 ± 0.0
	Total sleep time	7.6 ± 0.1	7.4 ± 0.1[a]	7.8 ± 0.1[b]
Short sleep	Time in bed	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
	Total sleep time	3.8 ± 0.0	3.8 ± 0.0	3.8 ± 0.0

All values are mean ± SEM. *p* values are derived from simple t-tests.

[a] and [b] indicate a significant difference in sleep time between men and women during habitual sleep with $p = 0.01$.

5.2 Energy and Food Intake Measures

The ad libitum energy and nutrient intake data during the 2 sleep conditions are presented in **Table 5.3**. Overall, daily caloric intake was 11.2% ($p = 0.31$) higher after short sleep (2807 ± 135 kcal/d) than that after habitual sleep (2525 ± 110 kcal/d), as **Figure 5.1** shows. The increases in energy intake in men and women, although not statistically significant, were 8.1% and 14.9%, respectively, compared to habitual sleep. Total fat, particularly saturated fat, played a major role in observed increments in energy

intake, as **Figure 5.2 & 5.3** show. Participants consumed more total ($p = 0.018$) and saturated fat ($p = 0.052$) after short sleep (111.7 $\pm$ 8.1 g and 36.7 $\pm$ 4.8 g, respectively) relative to habitual sleep (91.9 $\pm$ 5.8 g and 28.3 $\pm$ 2.6 g, respectively). This effect was more pronounced in women as they demonstrated 35.5% and 47.7% increases in fat ($p = 0.036$) and saturated fat ($p = 0.057$) intakes, respectively, after short sleep compared to habitual sleep, whereas sleep-deprived men had no changes in either total ($p = 0.29$) or saturated fat ($p = 0.59$) intakes. With regard to protein intake, participants tended to consume higher levels during the period of short sleep (97.8 $\pm$ 5.1 g) relative to habitual sleep (88.3 $\pm$ 3.5 g, $p = 0.09$). Carbohydrate intake, however, was not affected by sleep duration in all subjects ($p = 0.21$) or in participants stratified by gender ($p = 0.26$ vs. $p = 0.38$ in men vs. women, respectively).

Table 5.3 Energy and nutrient intakes during a period of habitual or short sleep by normal-weight adults

Subjects	Energy and nutrients	Habitual sleep	Short sleep	*p* values	Δ (%)
All (n = 26)	Energy, kcal/d	2525 ± 110	2807 ± 135	0.031*	11.2
	Total fat, g	91.9 ± 5.8	111.7 ± 8.1	0.018*	21.5
	Saturated fat, g	28.3 ± 2.6	36.7 ± 4.8	0.052	29.7
	CHO, g	346.2 ± 20.5	400.1 ± 40.9	0.21	15.6
	Protein, g	88.3 ± 3.5	97.8 ± 5.1	0.090	10.8
Men	Energy, kcal/d	2749 ± 189	2972 ± 170	0.15	8.1
(n = 13)	Total fat, g	95.0 ± 8.8	103.0 ± 6.9	0.29	8.4
	Saturated fat, g	26.0 ± 3.7	28.2 ± 3.5	0.59	8.5
	CHO, g	385.5 ± 36.3	420.1 ± 31.6	0.26	9.0
	Protein, g	97.0 ± 4.1	102.3 ± 8.1	0.48	5.5
Women	Energy, kcal/d	2300 ± 82	2642 ± 207	0.12	14.9
(n = 13)	Total fat, g	88.9 ± 7.8	120.5 ± 14.7	0.036*	35.5
	Saturated fat, g	30.6 ± 3.8	45.2 ± 8.5	0.057	47.7
	CHO, g	306.8 ± 12.9	380.1 ± 76.8	0.38	23.9
	Protein, g	79.7 ± 4.5	93.3 ± 6.3	0.11	17.1

All values are mean ± SEM. *p* values are derived from paired t-tests.

CHO, carbohydrate intake in gram.

"Δ" indicates percent increases in energy and nutrient intakes during short sleep compared to habitual sleep.

* indicates significant differences in energy and nutrient intakes between habitual and short sleep with $p \leq 0.05$.

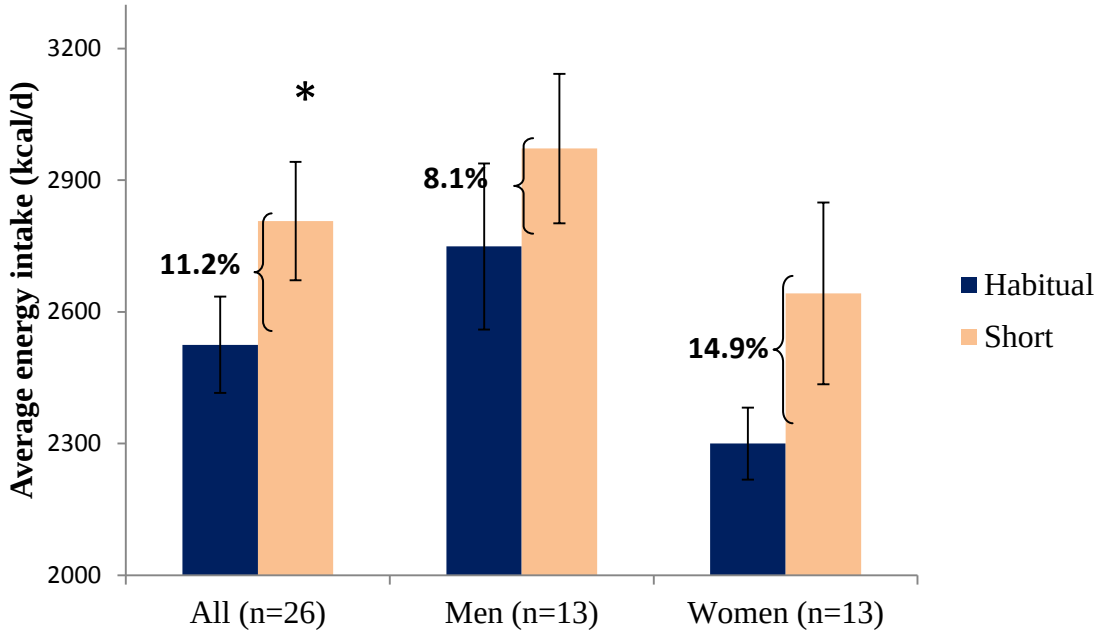

Figure 5.1 Energy intake during a period of habitual or short sleep by normal-weight adults

All values are mean ± SEM. *p* values are derived from paired t-tests.

* indicates significant differences between habitual and short sleep with $p \leq 0.05$.

" { " represents percent increases in energy intake during short sleep compared to habitual sleep.

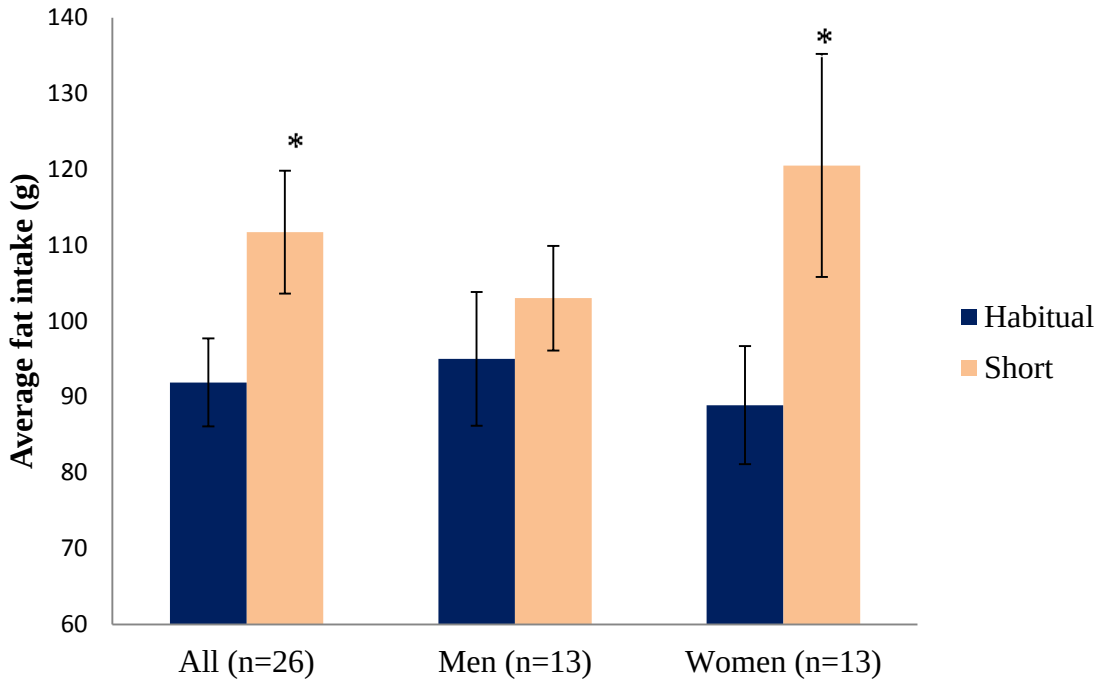

Figure 5.2 Total fat intake during a period of habitual or short sleep by normal-weight adults

All values are mean ± SEM. *p* values are derived from paired t-tests.

* indicates significant differences in total fat intake between habitual and short sleep with $p \leq 0.05$.

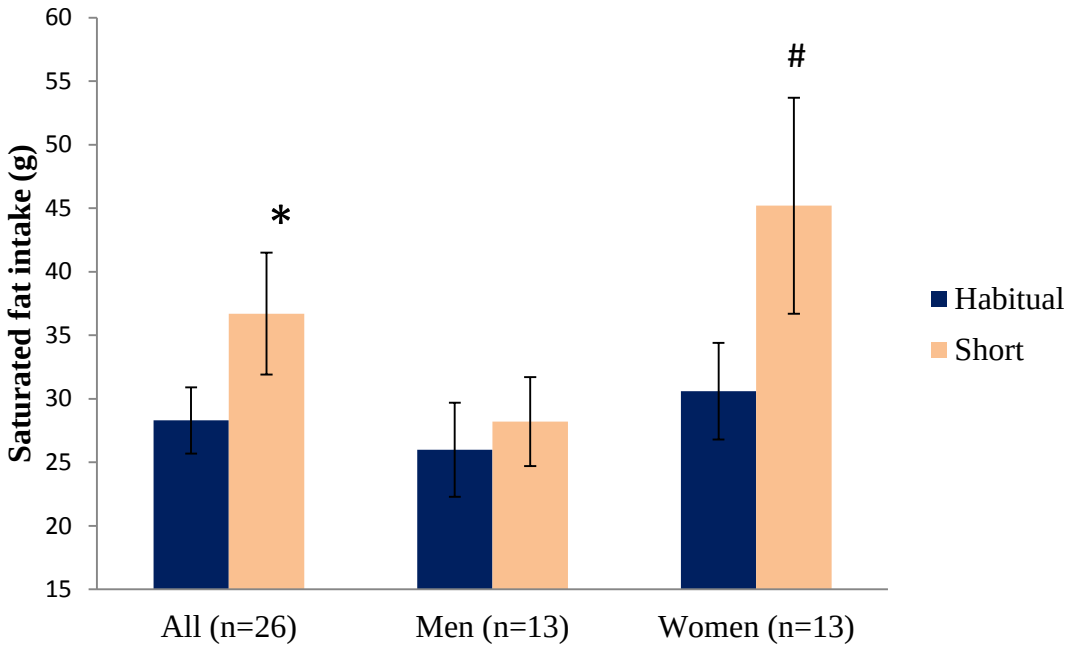

Figure 5.3 Saturated fat intake during a period of habitual or short sleep by normal-weight adults

All values are mean ± SEM. *p* values are derived from paired t-tests.

* indicates a trend of difference in saturated fat intake of all participants (n = 26) between habitual and short sleep with $p = 0.052$.

# indicates a trend of difference in saturated fat intake of women participants (n = 13) between habitual and short sleep with $p = 0.057$.

Percent energy (% energy) consumed as individual macronutrients is described in **Table 5.4**, showing trends for increased fat and saturated fat as % energy after sleep restriction. In all subjects, % energy from fat tended to be higher after short sleep (35.8 ± 1.8%) compared to habitual sleep (32.5 ± 1.5%, $p = 0.099$). Similarly, % energy taken as saturated fat after short sleep (11.3 ± 1.1%) tended to be greater relative to habitual sleep (10.0 ± 0.9%, $p = 0.14$). For women, particularly, % energy from fat ($p = 0.067$) and

saturated fat (p = 0.099) also tended to be greater after sleep restriction compared to habitual sleep duration, but the trend was not seen in men. Regarding % energy consumed as other macronutrients including carbohydrate and protein, no difference or tendency was observed between short and habitual sleep.

Table 5.4 Percent energy (% energy) taken as nutrients during a period of habitual or short sleep by normal-weight adults

Subjects	**Nutrients**	**% Energy**		***p* values**
		Habitual sleep	**Short sleep**	
All (n = 26)	Total fat	32.5 ± 1.5	35.8 ± 1.8	0.099
	Saturated fat	10.0 ± 0.9	11.3 ± 1.1	0.14
	CHO	54.7 ± 1.6	57.4 ± 6.2	0.66
	Protein	14.4 ± 0.6	14.3 ± 0.7	0.95
Men (n = 13)	Total fat	30.7 ± 2.1	31.5 ± 1.8	0.74
	Saturated fat	8.3 ± 1.0	8.5 ± 0.8	0.87
	CHO	55.8 ± 2.5	56.0 ± 2.2	0.95
	Protein	14.8 ± 0.9	13.9 ± 0.9	0.32
Women	Total fat	34.4 ± 2.2	40.1 ± 2.7	0.067
(n = 13)	Saturated fat	11.8 ± 1.3	14.2 ± 1.7	0.099
	CHO	53.6 ± 2.0	58.9 ± 12.5	0.67
	Protein	13.9 ± 0.7	14.7 ± 1.1	0.61

All values are mean ± SEM. p values are derived from paired t-tests.

CHO, percent energy taken from carbohydrate.

Eating occasions were also recorded during the ad libitum eating period, showing significant difference between short and habitual sleep **(Figure 5.4)**. It is clear that subjects ate more times during short sleep (6.1 $\pm$ 0.3 times/d) compared to habitual sleep (5.0 $\pm$ 0.2 times/d, $p < 0.0001$) with 20 out of 26 participants increasing their eating frequencies. A 1 time/d minimum increase in eating occasions was equally observed in both men ($p = 0.0028$) and women ($p = 0.005$). According to eating time recorded by research personnel, 7 participants ate after 10 p.m. during short sleep.

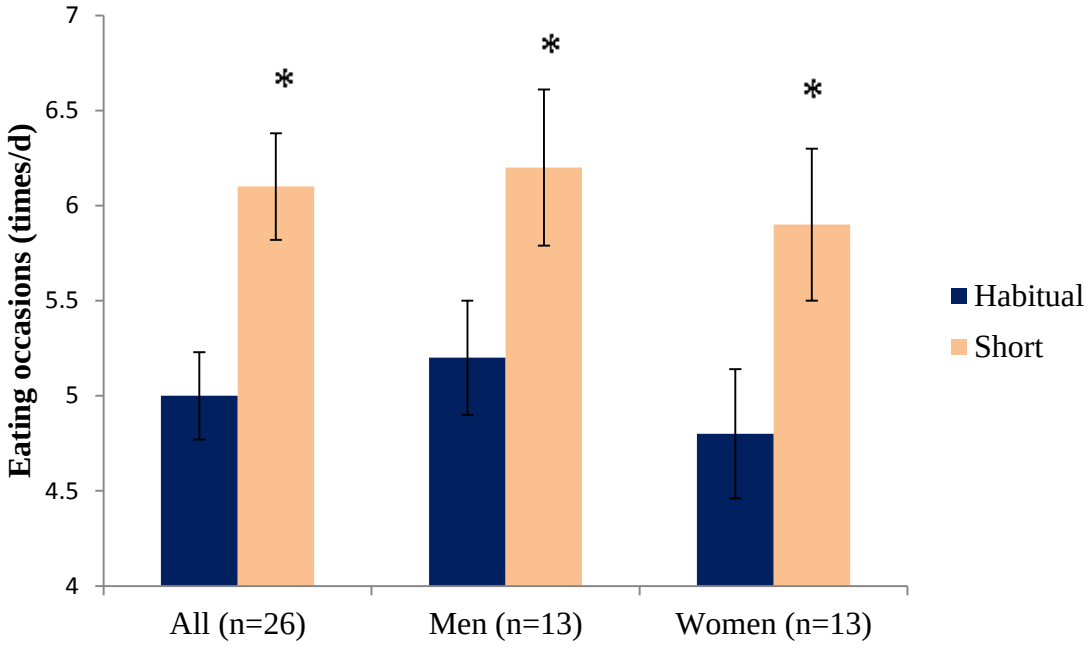

Figure 5.4 Eating occasions during a period of habitual or short sleep in normal-weight adults

All values are mean $\pm$ SEM. p values are derived from paired t-tests.

* indicates significant differences in eating occasions between habitual and short sleep with $p \leq 0.05$.

5.3 Resting Metabolic Rate Measures

There was no difference observed in RMR between habitual sleep (1521 ± 33 kcal/d) and short sleep (1489 ± 34 kcal/d, $p = 0.13$), or respiratory quotient (0.79 ± 0.007 vs. 0.79 ± 0.006, habitual vs. short sleep, respectively, $p = 0.61$). There was no significant difference in RMR and RQ between the 2 phases across gender **(Table 5.5)**.

Table 5.5 Average resting metabolic rate and respiratory quotient measured by indirect calorimetry during a period of habitual or short sleep by normal-weight adults

Subjects	Parameters	Habitual sleep	Short sleep	*p* values
All (n = 26)	RMR, kcal/d	1521 ± 33	1489 ± 34	0.13
	RQ	0.79 ± 0.007	0.79 ± 0.006	0.61
Men (n = 14)	RMR, kcal/d	1628 ± 33	1590 ± 37	0.23
	RQ	0.80 ± 0.009	0.80 ± 0.009	1.0
Women	RMR, kcal/d	1395 ± 33	1372 ± 38	0.41
(n = 13)	RQ	0.79 ± 0.012	0.78 ± 0.007	0.51

All values are mean ± SEM. *p* values are derived from paired t-tests.

RMR, resting metabolic rate

RQ, respiratory quotient.

5.4 Energy Expenditure and Physical Activity Level Measures

Individual TEE during habitual and short sleep is shown in **Figure 5.5 & 5.6** for men and women, respectively. Overall, total energy expended over a 6-d period of short sleep (2493 ± 87 kcal/d) was not different from that during habitual sleep (2495 ± 113 kcal/d, $p = 0.99$), in either men ($p = 0.83$) or women ($p = 0.85$) (**Table 5.6)**. Similarly, no differences were observed in TEE relative to individual body weight between the two

sleep conditions in all subjects (p = 0.99) or in separate analyses of men (p = 0.77) and women (p = 0.83).

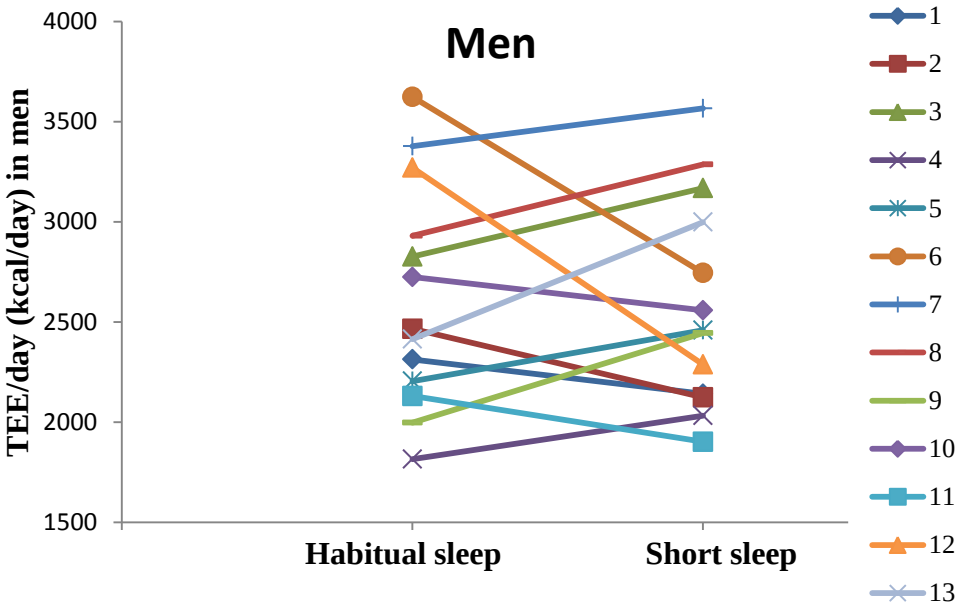

Figure 5.5 Individual total energy expenditure during a period of habitual or short sleep by normal-weight men (n = 13)

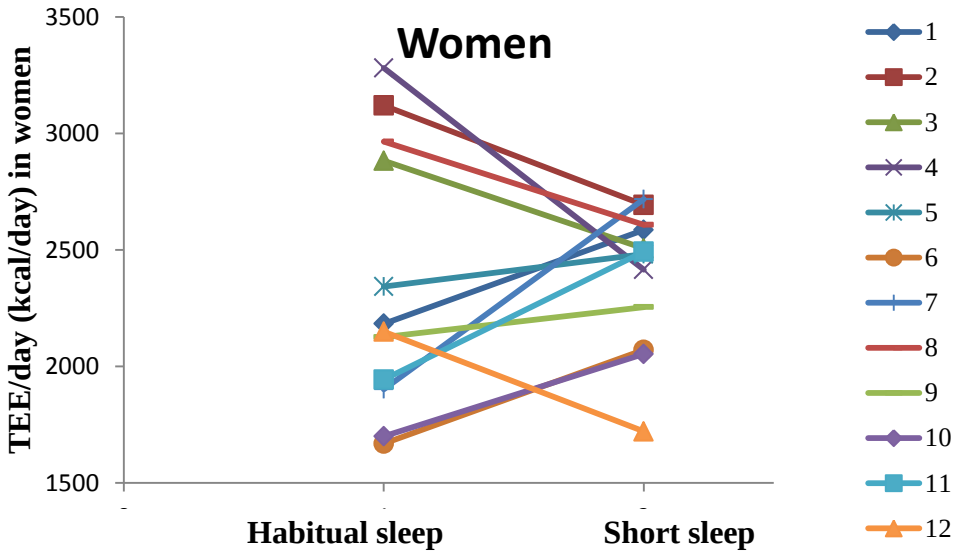

Figure 5.6 Individual total energy expenditure during a period of habitual or short sleep by normal-weight women (n = 12)

Table 5.6 Average total energy expenditure and relative to body weight during a period of habitual and short sleep by normal-weight adults

Subjects	Parameters	Habitual sleep	Short sleep	*p* values
All	TEE, kcal/d	2495 ± 113	2493 ± 87	0.99
(n = 25)	TEE/BW, kcal/kg/d	36.3 ± 1.6	36.3 ± 1.2	0.99
Men	TEE, kcal/d	2623 ± 155	2594 ± 145	0.83
(n = 13)	TEE/BW, kcal/kg/d	34.5 ± 1.9	34.0 ± 1.8	0.77
Women	TEE, kcal/d	2356 ± 163	2384 ± 87	0.85
(n = 12)	TEE/BW, kcal/kg/d	38.3 ± 2.6	38.8 ± 1.2	0.83

All values are mean ± SEM. *p* values are derived from paired t-tests.

TEE, total energy expenditure.

TEE/BW, total energy expenditure relative to body weight.

Mean PAL values were not different between habitual (1.66 ± 0.08) and short sleep (1.69 ± 0.06, $p = 0.70$) (**Table 5.7**). These PAL values generally represented a moderate activity level in participants (WHO, 2000; FAO/WHO/UNU, 2001). No effects of sleep duration on PAL were observed in men ($p = 0.75$) or women ($p = 0.82$). In agreement with PAL data, AEE used for physical activity was not different between habitual (741.0 ± 97.4 kcal/d) and short sleep (758.9 ± 76.6 kcal/d, $p = 0.85$). Men ($p = 0.86$) and women ($p = 0.95$), separately, showed no difference in AEE between the 2 sleep phases.

Table 5.7 Average physical activity level and activity energy expenditure during a period of habitual or short sleep by normal-weight adults

Subjects	Parameters	Habitual sleep	Short sleep	*p* values
All (n = 24)	PAL	1.66 ± 0.08	1.69 ± 0.06	0.70
	AEE, kcal/d	741.0 ± 97.4	758.9 ± 76.6	0.85
Men (n = 12)	PAL	1.61 ± 0.10	1.65 ± 0.09	0.75
	AEE, kcal/d	732.1 ± 134.4	756.3 ± 123.6	0.86
Women	PAL	1.71 ± 0.12	1.74 ± 0.09	0.82
(n = 12)	AEE, kcal/d	751.5 ± 148.2	762.0 ± 88.2	0.95

All values are mean ± SEM. *p* values are derived from paired t-tests.

PAL, physical activity level.

AEE, activity energy expenditure.

5.5 Energy Balance and Daily Body Weight Measures

Because both controlled and ad libitum feedings were employed in each impatient phase, the best way to describe the effect of sleep duration on energy balance,

concomitantly on body weight, is according to individual time intervals. During the first 4 d, subjects were in a state of negative energy balance (-402.9 ± 108.7 kcal/d vs. -437.7 ± 88.5 kcal/d during habitual vs. short sleep, respectively, $p = 0.72$) due to the controlled diets **(Figure 5.7)**. Therefore, mean body weights were reduced by 0.99 ± 0.22 kg (1.4%) and 0.93 ± 0.24 kg (1.3%) from d 1 to d 4 during habitual and short sleep, respectively ($p = 0.81$). The observed reduction in body weight during this interval was more remarkable, although not significantly different, in men (-1.37 ± 0.30 kg vs. -1.22 ± 0.39 kg during habitual vs. short sleep, respectively) than women (-0.65 ± 0.30 kg vs. -0.64 ± 0.28 kg during habitual vs. short sleep, respectively).

On d 5, self-selected foods and beverages eaten by subjects were completely recorded and measured, showing opposite results to the previous 4 d. In comparison with the first interval with controlled diets, the total energy intake increased by 552.2 ± 87.7 kcal/d ($p < 0.0001$) and 822.8 ± 141.3 kcal/d ($p < 0.0001$) during habitual and short sleep, respectively **(Table 5.8)**. With the same mean energy outcome per day, therefore, energy balance during habitual and short sleep both became positive on d 5 (149.3 ± 146.7 kcal/d and 385.0 ± 175.5 kcal/d, respectively, $p = 0.10$), as shown in **Figure 5.8**. The 1-d positive energy balance was then accompanied by an augment in body weight from d 5 to d 6 (0.32 ± 0.062 kg vs. 0.45 ± 0.11 kg after habitual vs. short sleep, respectively, $p = 0.23$) **(Table 5.9)**. Positive energy balance and weight gain were seen in men and women on d 5 compared with previous days during both sleep phases.

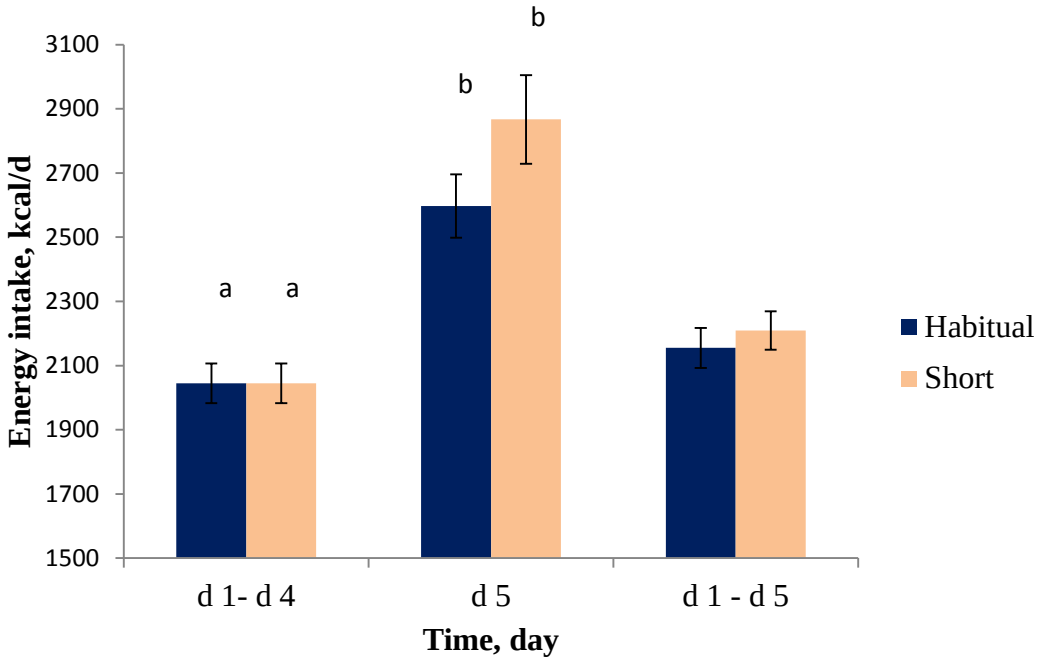

Figure 5.7 Average energy intake during d 1 to d 4, d 5 and d 1 to d 5 of habitual or short sleep by normal-weight adults (n = 24)

All values are mean ± SEM. *p* values are derived from paired t-tests.

a and b indicate significant differences in energy intake between first 4 d of controlled feeding and ad libitum feeding on d 5 during habitual and short sleep with $p \leq 0.0001$.

Table 5.8 Average energy balance, energy intake vs. total energy expenditure, during 5 d of habitual or short sleep by normal-weight adults in three time intervals

Subjects	Parameters[1], kcal/d	Habitual sleep	Short sleep	*p* values
All (n = 24)	EI^{1-4}	2045 ± 62[a]		
	EI^{5}	2597 ± 99[b]	2867 ± 138[b]	0.053
	$EI^{5} - EI^{1-4}$	552.2 ± 87.7	822.8 ± 141.3	0.053
	EI^{1-5}	2155 ± 62	2209.2 ± 60.3	0.053
	TEE	2448 ± 108	2482 ± 90	0.72
	EB^{1-4}	-402.9 ± 108.7	-437.7 ± 88.5	0.72
	EB^{5}	149.3 ± 146.7	385.0 ± 175.5	0.10
	EB^{1-5}	-292.5 ± 111.9	-273.2 ± 96.0	0.83
Men (n = 12)	EI^{1-4}	2290 ± 58[a]		
	EI^{5}	2894 ± 132[b]	3078 ± 145[b]	0.25
	$EI^{5} - EI^{1-4}$	603.5 ± 145.9	787.7 ± 162.3	0.25
	EI^{1-5}	2411 ± 53	2448 ± 52	0.25
	TEE	2539 ± 142	2581 ± 157	0.75
	EB^{1-4}	-249.1 ± 138.6	-290.6 ± 148.0	0.75
	EB^{5}	354.4 ± 216.1	497.1 ± 203.5	0.38
	EB^{1-5}	-128.4 ± 145.9	-133.1 ± 146.9	0.76
Women	EI^{1-4}	1799 ± 39[a]		
(n = 12)	EI^{5}	2300 ± 89[b]	2657 ± 224[b]	0.14
	$EI^{5} - EI^{1-4}$	500.8 ± 101.7	857.8 ± 238.7	0.14
	EI^{1-5}	1899 ± 33	1971 ± 56	0.14
	TEE	2356 ± 163	2384 ± 87	0.85
	EB^{1-4}	-556.7 ± 161.0	-584.8 ± 83.1	0.85
	EB^{5}	-55.9 ± 188.9	273.0 ± 291.6	0.18
	EB^{1-5}	-456.6 ± 161.9	-413.3 ± 115.8	0.97

All values are mean ± SEM. *p* values are derived from paired t-tests.
[1] EI^{1-4}, average energy intake from d 1 to d 4; EI^{5}, average EI on d 5; EI^{1-5}, average EI from d 1 to d 5; TEE, average total energy expenditure; EB^{1-4}, average energy balance from d 1 to d 4; EB^{5}, average EB on d 5; EB^{1-5}, average EB from d 1 to d 5.
a and b indicate significant differences in energy intake between first 4 d of controlled feeding and ad libitum feeding on d 5 during habitual and short sleep with $p \leq 0.05$.

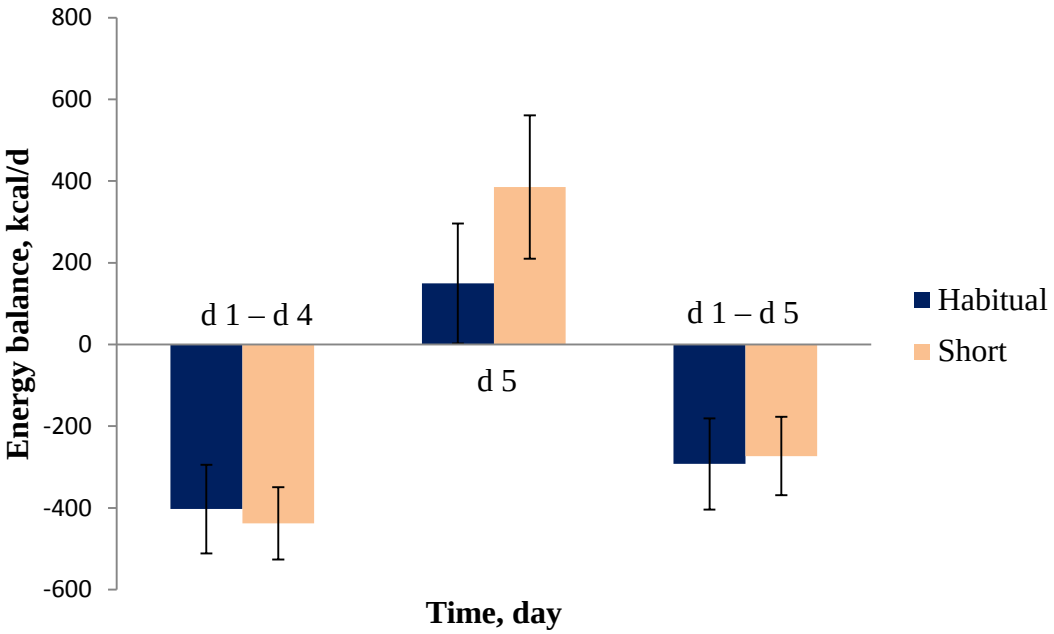

Figure 5.8 Average energy balance during d 1 to d 4, d 5 and d 1 to d 5 of habitual or short sleep by normal-weight adults (n = 24)

All values are mean ± SEM.

Table 5.9 Daily body weight of participants during a period of habitual or short sleep

Subjects	Day[1]	Daily body weight, kg		*p* value
		Habitual sleep	Short sleep	
All	d 1	69.60 ± 2.17	69.65 ± 2.26	0.85
(n = 24)	d 2	68.92 ± 2.17	69.51 ± 2.22	0.0057
	d 3	68.91 ± 2.13	69.22 ± 2.21	0.14
	d 4	69.00 ± 2.15	69.05 ± 2.23	0.76
	d 5	68.63 ± 2.11	68.71 ± 2.17	0.65
	d 6	68.93 ± 2.14	69.17 ± 2.21	0.15
	d 5 – d 1	-0.99 ± 0.22 (-1.4%)	-0.93 ± 0.24 (-1.3%)	0.81
	d 6 – d 5	0.32 ± 0.062 (0.4%)	0.45 ± 0.11 (0.6%)	0.23
	d 6 – d 1	-0.66 ± 0.19 (-0.9%)	-0.48 ± 0.17 (-0.7%)	0.33
Men	d 1	76.85 ± 2.82	77.17 ± 2.97	0.28
(n = 12)	d 2	76.08 ± 2.88	76.88 ± 2.91	0.014
	d 3	75.93 ± 2.84	76.53 ± 2.87	0.051
	d 4	76.00 ± 2.88	76.28 ± 2.90	0.20
	d 5	75.51 ± 2.81	75.93 ± 2.79	0.14
	d 6	76.00 ± 2.83	76.50 ± 2.87	0.025
	d 5 – d 1	-1.37 ± 0.30 (-1.8%)	-1.22 ± 0.39 (-1.5%)	0.68
	d 6 – d 5	0.50 ± 0.077 (0.7%)	0.58 ± 0.20 (0.7%)	0.70
	d 6 – d 1	-0.84 ± 0.27 (-1.1%)	-0.64 ± 0.27 (-0.8%)	0.46
Women	d 1	62.36 ± 1.49	62.13 ± 1.50	0.58
(n = 12)	d 2	61.76 ± 1.47	62.14 ± 1.51	0.19
	d 3	61.90 ± 1.43	61.90 ± 1.57	1.00
	d 4	61.98 ± 1.42	61.73 ± 1.62	0.40
	d 5	61.74 ± 1.47	61.50 ± 1.55	0.36
	d 6	61.86 ± 1.46	61.83 ± 1.55	0.92
	d 5 – d 1	-0.65 ± 0.30 (-1.0%)	-0.64 ± 0.28 (-1.0%)	0.92
	d 6 – d 5	0.13 ± 0.064 (0.2%)	0.33 ± 0.10 (0.6%)	0.11
	d 6 – d 1	-0.48 ± 0.26 (-0.8%)	-0.31 ± 0.20 (-0.5%)	0.55

All values are mean ± SEM. *p* values are derived from paired t-tests.

[1] Daily body weight was measured first in the morning after subjects woke up, representing the ultimate weight after energy income and outcome in previous days.

"( )" represents percent changes in body weight during specific time intervals.

Over the course of a 6-d study period, although ad libitum energy intake was diluted by the previous 4-d controlled feeding, the average energy intake during short sleep (2209 ± 60 kcal/d) was still higher than that during habitual sleep (2155 ± 62 kcal/d, $p = 0.053$) **(Table 5.8)**. Regarding the overall energy balance, subjects on average had negative energy balance (-292.5 ± 111.9 kcal/d vs. -273.2 ± 96.0 kcal/d during habitual vs. short sleep, respectively, $p = 0.83$), as shown in **Figure 5.8**. Concomitantly, body weights of participants on average decreased by 0.66 ± 0.19 kg (0.9%) during the 6-d period of habitual sleep and 0.48 ± 0.17 kg (0.7%) during short sleep **(Table 5.9)**. However, no pronounced effects of sleep duration on energy balance or change in body weight were observed in all participants, or in men and women examined separately.

Chapter 6: Discussion and Conclusion

The main findings of the present study conducted in normal-weight young adults indicate that 5 consecutive nights of sleep restriction has an impact on food and energy consumption, but not on energy expenditure or physical activity levels. The increasing effect of short sleep on food intake, particularly fat intake, was more remarkable in women than men. Overall, no effects of sleep duration on energy balance or changes in body weight were detected. However, the additional energy intake during short sleep was not counteracted by raised TEE, which theoretically would lead to weight gain over a longer term.

Ad libitum energy intake after sleep restriction was significantly higher than that after habitual sleep, accompanied by increased fat intake and elevated eating occasions. This result is consistent with some recently published studies in humans (Spiegel *et al.*, 2004; Brondel *et al.*, 2010; Nedeltcheva *et al.*, 2009; Bosy-Westphal *et al.*, 2008). The extra calorie intake after short sleep was approximately 300 kcal/d on average, which is similar to the observation by Nedeltcheva *et al.* (2009), but less than that observed by Bosy-Westphal *et al.* (2008) (20%) and Brondel *et al.* (2010) (22%). These discrepancies may be mainly attributed to variability in study design, including the length of intervention periods, the degree of eating freedom, and the method of estimating food and energy intakes. It is noted that our measurements of ad libitum food and energy intakes took place after 4 d of interventions in sleep time, 9 h and 4 h, whereas Brondel *et al.* (2010) reported energy intake after a single night of reduced sleep. Also, Bosy-Westphal *et al.* (2008) employed a 4-night period with gradually increased sleep curtailment (7 h, 6 h, 6 h and 4h sleep/night) right after habitual sleep (> 8 h/night) without a washout period.

Moreover, participants in our study had full freedom to purchase their own foods and beverages, and eat ad libitum, whereas previous studies customized buffet-like meals and established meal times which limited food availability and eating occasions to some extent (Brondel *et al.*, 2010; Nedeltcheva *et al.*, 2009; Schmid *et al.*, 2009). In addition, food intake was carefully weighed and recorded by our staff, whereas other studies relied on self-reports (Brondel *et al.*, 2010; Bosy-Westphal *et al.*, 2008) which can be variable and inaccurate depending on study populations (Garcia-Dominic *et al.*, 2010). In contrast, Schmid *et al*. (2009) observed no increase in food intake after a single night of 4-h sleep compared with 8-h regular sleep in 15 healthy men. However, other than variable study design and a relatively small sample size, most importantly, all subjects investigated were men who may have different susceptibility to hyperphagia after acute sleep curtailment compared to women, which was demonstrated by women having a greater increase (14.9%) in calorie intake than men (8.1%) in our study.

The observed increase in food intake was induced by a rise in fat, particularly saturate fat intake, after reduced sleep in our study. Similarly, a large cross-sectional study concluded that sleep duration (< 7 h and 7-9 h/night) was inversely associated with total energy intake, particularly the fat intake in 2,828 Chinese people (Shi *et al.*, 2008). This altered eating pattern, if sustained, may predispose people to a higher risk of diseases induced by excessive fat and/or caloric intake, such as obesity and cardiovascular disease. In fact, the mean saturated fat intake measured was 11.3% of energy intake during short sleep and 10.0% during habitual sleep. Both of these values are much higher than the recommendation made by American Heart Association that saturated fat consumption should not exceed 7% of total caloric intake/d (Lichtenstein *et*

al., 2006). However, after converting fat and saturated fat intakes in gram to % energy, the significance disappeared. The result is similar to those of Bosy-Westphal *et al.* (2008) and Schmid *et al.* (2009) who found no changes in % energy from fat or any other nutrients after reduced sleep in healthy women and men, but contrasts to the result of Brondel *et al.* (2010) that a 7.9% increase in % energy from fat after one night of 4-h sleep restriction.

Consistent with many clinical studies (Brondel *et al.*, 2010; Nedeltcheva *et al.*, 2009; Bosy-Westphal *et al.*, 2008; Schmid *et al.*, 2009), we did not find any effect of sleep duration on carbohydrate or protein intakes, either presented in gram or % energy, which may be due to a single day of food recording. However, Nedeltcheva *et al.* (2009) in their results pointed out that a 14-d period of 5.5-h sleep was accompanied by elevated calorie intake from snacks ($p = 0.026$), particularly with higher carbohydrate content ($p = 0.04$), without significant changes in energy, macronutrients, or macronutrient distribution from meals. It is possible that their strict settings of meal times and customized meals accompanied by unlimited access to snacks predisposed subjects to excess snack intake when they had extended waking hours, especially during the night hours of short sleep. Moreover, their study included men and women (n = 11) who were sedentary with a mean BMI of 26.5 kg/m^2. The relatively small sample size, and variable inclusion criteria in terms of baseline lifestyle and BMI may partially explain some variability in dietary results (Nedeltcheva *et al.*, 2009).

Our study also found that the eating occasion increased during short sleep. This is expected because the extra waking hours resulting from sleep curtailment not only prolonged conscious time to experience hunger or boredom, but also extended exposure

to palatable foods. Furthermore, 6 subjects were observed eating after 10 p.m. during short sleep. The alteration in feeding behavior may enhance the susceptibility to night eating syndrome which is characterized by morning anorexia, evening hyperphagia and insomnia (O'Reardon *et al.*, 2005). As sleep-obesity research indicated, nonhomeostatic factors including eating at night shortly before sleep play a considerable role in weight gain (Saper *et al.*, 2002).

Our energy expenditure data show no significant differences in TEE and its components, including RMR and AEE, between short and habitual sleep, which indicates that participants, while having longer waking hours, may expend same amount of energy for resting, voluntary and involuntary activities as during habitual sleep. This finding is in accordance with results found by Bosy-Westphal *et al.* (2008) and Nedeltcheva *et al.* (2009) using continuous 24-h heart rate monitors and the doubly labeled water method, respectively. Moreover, our estimated PAL values, in line with AEE results, were not different between short and habitual sleep, indicating that participants during short sleep had similar levels of activity engagement as that during habitual sleep. However, some studies reported either a reduction or an increase in physical activity with sleep restriction. In Schmid *et al.* study (2009), for example, they reported a reduction in physical activity and a shift toward lower-intensity activities, measured by wrist accelerometers, after a single night of sleep restriction in 15 young men. On the other hand, another recent study noted an increase in physical activity, assessed by actimeters, after 2 d of sleep restriction in 12 young men (Brondel *et al.*, 2010). As introduced before, accelerometers are insensitive to static work or upper body movement, so they do not represent whole-body physical activity and particularly are unreliable to detect TEE (Plasqui & Westerterp,

2007). Therefore, the lack of precision in energy expenditure assessments could be one of limitations of the measurements. Moreover, the relatively short sleep intervention periods, small sample sizes, and specification in men may reduce the reliability of their findings. In contrast, our study applied 6 d of sleep restriction to 30 men and women whose TEEs were directly measured by the gold standard DLW method, which has shown more reliable and conclusive results concerning energy expenditure and physical activity.

Combining energy expenditure and dietary intake data, effects of sleep duration on energy balance and changes in body weight were not identified statistically. However, in comparison with habitual sleep, the energy balance under ad libitum eating conditions tended to be more positive after short sleep, accompanied by slightly greater magnitude of weight gain. Unfortunately, very few clinical trials have considered or reported energy balance and daily body weight data, probably due to the extremely short length of study period. However, many large-scale and epidemiological studies on sleep-obesity association, although unable to directly measure individual energy balance, reported abundant data on the inverse relationship between sleep duration and weight or BMI across age, gender and countries. Bawazeer *et al.* (2009), for example, studied a total of 5,877 Saudi Arabian children aged 10-19 y and reported that the odds of obesity, as a function of <7 h of sleep, was 1.28 and 1.38 in boys and girls, respectively. In a cross-sectional and longitudinal analysis of 9,588 American adults aged 32-49 y, sleep durations under 7 h were consistently associated with increased likelihood of obesity (Gangwisch *et al.*, 2005). A meta-analysis of short sleep duration and obesity in children and adults also supported these findings (Cappuccio *et al.*, 2008). Based on those previous studies, it is possible to hypothesize that the differential magnitude of gained

weight between short and habitual sleep in our study may be enlarged as ad libitum eating duration is prolonged, but this requires more detailed human studies.

The earlier hypothesis also elicits some limitations of the present study worth noting. First, we only had food intake data from a single day of ad libitum eating, which immediately followed a period of controlled feeding. The representativeness of these 1-d food and energy intake data might be questioned. Multiple days food records are typically necessary for participant self-report to minimize daily variability in food intakes. We, however, are confident about the quality of our data because subjects were under constant supervision and study personnel were responsible for food provision and data recording. The pre-measurement, controlled feeding also has the advantage that our results are not adulterated by serendipitous differences in pre-measurement feeding status. Unlike our study, many clinical trials allow subjects to eat freely without an adaptation period of controlled feeding while the sleep intervention is ongoing, and then immediately measured energy intake, energy expenditure and hormone changes (Nedeltcheva *et al.*, 2009; Bosy-Westphal *et al.*, 2008; Schmid *et al.*, 2009), which raises a question of whether the observed effects result solely from sleep interventions or a combination of other factors, such as environmental, eating, and food intake-induced hormone alterations.

Secondly, participants were young with normal BMI. It is unknown whether or not depriving sleep in older or overweight/obese subjects would evoke more pronounced metabolic responses observed in the present study. Our participants with overall negative energy balance across the study periods had no difference in weight loss under sleep restriction compared with habitual sleep. This observation is further clarified in a recent study by Nedeltcheva *et al.* (2010) who found that in presence of dietary restriction,

sleep-deprived people, although they lost same amounts of weight as normal sleep people, they experienced a compromised efficacy of dietary efforts to reduce weight. In the randomized crossover study, 10 overweight and slightly obese (BMI, 25-32 kg/m^2) adults aged 35-49 y spent two 14-d periods as inpatients with 8.5 h or 5.5 h per night of sleep and were given calorie-restricted diets. Similar weights were lost with the 2 sleep treatments, but sleep curtailment decreased the proportions of weight loss as fat and promoted the loss of fat-free mass. Both of our studies did not find significant effects of sleep curtailment on energy expenditure or weight changes, but the potential to compromise dietary efforts to reduce weight and to increase the risk of overweight and obesity in normal- or heavy-weight people is not inconceivable. Further studies are required to examine the longer-term effects of sleep deprivation on energy metabolism and body weight and composition.

Although our findings provide important insight into the affected energy metabolism as a function of reduced sleep, further exploration is certainly necessary. First, the direct effects of short sleep on body composition, substrate utilization, and hormones should be explored, which may be helpful to the improvement of prevention and treatment of overweight or obesity. Second, investigations of the dose-response correlation between decreased/increased sleep hours and elevated/reduced body weight in humans will facilitate consultants to provide health information.

In conclusion, normal-weight young and healthy adults exposed to 4 consecutive nights of sleep restriction can have energy metabolism disrupted via increased energy intake particularly from fat. The elevation in caloric intake may also be accompanied by increased eating occasions since the waking hours are prolonged. However, because

energy expenditure remains unaffected as a function of sleep duration, the increased caloric intake may not be compensated by a rise in energy expenditure. Therefore, chronic sleep curtailment may induce excessive energy intake without being balanced by raised energy expenditure, which may lead to positive energy balance over time and increase the risk of weight gain and/or obesity.

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Appendices

Appendix I Appendix Tables

Table 1 Male anthropometric data and intermediate parameters estimated for energy expenditure calculations during a period of habitual or short sleep (n = 13)

Phase	Sleep L/S	ID	Age y	Height kg	Weight cm	BMI kg/m^2	k_h	k_o	r_{CO2} L/d	TEE kcal/d	TEE/BW kcal/kg/d	RMR kcal/d	AEE kcal/d
1	L	AL12	30	179.4	80.2	24.9	0.15	0.19	406.7	2313.2	28.8	1694	387.88
2	S	AL12	30	179.4	79.4	24.7	0.15	0.18	376.6	2142.1	27.0	1711	216.89
1	S	BT18	32	167.6	58	20.6	0.12	0.14	373.4	2124.2	36.6	1379	532.78
2	L	BT18	32	167.7	58.8	20.9	0.11	0.13	433.6	2466.2	41.9	1595	624.58
1	L	CH19	32	187.5	90.8	25.8	0.11	0.13	496.9	2826.6	31.1	1795	748.94
2	S	CH19	32	187.8	92.4	26.2	0.13	0.15	556.9	3168.0	34.3	1784	1067.2
1	S	FA13	45	176.4	72.2	23.2	0.094	0.11	357.3	2032.6	28.2	1584	245.34
2	L	FA13	45	176.4	70.9	22.8	0.11	0.12	319.2	1815.9	25.6	1519	115.31
1	S	GB17	33	184.4	87	25.6	0.18	0.20	432.3	2459.0	28.3	1390	823.1
2	L	GB17	33	184.4	86.4	25.4	0.16	0.18	387.6	2204.7	25.5	1545	439.23
1	S	GF02	42	182.5	81.4	24.4	0.099	0.12	482.5	2744.8	33.7	1561	909.32
2	L	GF02	42	183.3	80	23.8	0.13	0.16	637	3623.2	45.3	1603	1657.88
1	L	HE08	35	174.9	79.6	26	0.16	0.18	593.8	3377.4	42.4	1893	1146.66
2	S	HE08	35	174.1	78.6	25.9	0.22	0.25	626.9	3566.2	45.4	1621	1588.58
1	S	JD07	35	176.7	71.2	22.8	0.13	0.14	577.7	3286.2	46.2	1563	1394.58
2	L	JD07	35	176.9	70.8	22.6	0.14	0.16	515.1	2930.2	41.4	1599	1038.18
1	S	JD40	30	180.6	69.2	21.2	0.092	0.12	429.8	2444.8	35.3	1628	572.32
2	L	JD40	30	180.9	69.4	21.2	0.099	0.12	351.3	1998.0	28.8	1679	119.2
1	L	ML48	44	185.8	85	24.6	0.072	0.093	479	2724.7	32.1	1757	695.23
2	S	ML48	44	186	84.4	24.4	0.08	0.10	449.8	2558.4	30.3	1805	497.56
1	L	PA27	35	166.8	63.4	22.8	0.076	0.097	374.6	2131.1	33.6	1522	395.99
2	S	PA27	35	166.9	65.4	23.5	0.086	0.11	334.5	1902.8	29.1	1447	265.52
1	S	RL11	43	185.4	81.8	23.8	0.15	0.17	402.2	2288.0	28.0	1511	548.2
2	L	RL11	43	185.4	80.6	23.4	0.143	0.17	574.8	3269.8	40.6	1435	1507.82
1	S	SN15	45	179.1	76.2	23.8	0.095	0.12	527.1	2998.2	39.3	1528	1170.38
2	L	SN15	45	179.1	76.6	23.9	0.13	0.15	424.5	2414.4	31.5	1533	639.96

L/S, habitual and short sleep, respectively; BMI, body mass index; k_h and k_o, turnovers of ^2H and ^{18}O isotopes; r_{CO2}, CO_2 production rate; TEE, total energy expenditure; TEE/BW, total energy expenditure relative to body weight; RMR, resting metabolic rate measured by indirect calorimetry; AEE, activity energy expenditure.

Table 2 Female anthropometric data and intermediate parameters estimated for energy expenditure calculations during a period of habitual or short sleep (n = 12)

Phase	Sleep L/S	ID	Age y	Height cm	Weight kg	BMI kg/m^2	k_h	k_o	r_{CO2} L/d	TEE kcal/d	TEE/BW kcal/kg/d	RMR kcal/d	AEE kcal/d
1	L	AH21	32	171.4	70	23.8	0.13	0.16	383.8	2183.2	31.2	1411	553.88
2	S	AH21		171.4	70.6	24.0	0.12	0.15	454.6	2586.1	36.6	1174	1153.49
1	L	CC51	31	179.2	72.4	22.5	0.087	0.11	548.6	3120.5	43.1	1540	1268.45
2	S	CC51		178.7	71.8	22.5	0.11	0.14	473.6	2694.0	37.5	1528	896.6
1	S	CO57	30	164.9	62.4	22.9	0.18	0.22	440.7	2506.9	40.2	1536	720.21
2	L	CO57		164.9	60.2	22.1	0.17	0.21	506.9	2883.5	47.9	1526	1069.15
1	S	CT47	30	158	58.2	23.3	0.21	0.25	424.7	2415.6	41.5	1227	947.04
2	L	CT47		157.7	57.4	23.1	0.17	0.22	576.9	3281.6	57.2	1220	1733.44
1	L	EL39	32	162.4	59.8	22.7	0.15	0.17	411.9	2343.2	39.2	1509	599.88
2	S	EL39		162.3	59.4	22.6	0.15	0.18	436.4	2482.6	41.8	1400	834.34
1	L	KC38	34	161.4	56.2	21.6	0.16	0.19	293.4	1669.0	29.7	1302	200.1
2	S	KC38		161.9	55.8	21.3	0.15	0.18	364.2	2071.5	37.1	1412	452.35
1	S	MC04	34	153.9	62.2	26.3	0.098	0.13	477.9	2718.4	43.7	1232	1214.56
2	L	MC04		153.9	62	26.2	0.13	0.15	334.4	1902.0	30.7	1293	418.8
1	L	OF53	44	160.9	62.2	24.0	0.12	0.15	521.3	2965.5	47.7	1548	1120.95
2	S	OF53		160.7	61.6	23.9	0.16	0.19	458.5	2608.2	42.3	1598	749.38
1	L	PS37	32	163.6	61	22.8	0.067	0.094	373.8	2126.2	34.9	1373	540.58
2	S	PS37		163.7	60.4	22.5	0.39	0.44	396.4	2254.7	37.3	1393	636.23
1	L	SI26	32	164.9	56.2	20.7	0.11	0.13	298.9	1700.2	30.3	1423	107.18
2	S	SI26		164.2	54.6	20.3	0.12	0.15	361.0	2053.7	37.6	1304	544.33
1	S	TL09	41	165	59.2	21.7	0.074	0.096	438.2	2492.8	42.1	N.A.	N.A.
2	L	TL09		164.9	59.8	22.0	0.097	0.12	341.8	1944.1	32.5	N.A.	N.A.
1	S	VC50	34	166.3	60.8	22.0	0.1	0.12	302.6	1721.5	28.3	1316	233.35
2	L	VC50		167.1	60.8	21.8	0.083	0.11	377.9	2149.6	35.4	1281	653.64

L/S, habitual and short sleep, respectively; BMI, body mass index; k_h and k_o, turnovers of ^{2}H and ^{18}O isotopes; r_{CO2}, CO_2 production rate; TEE, total energy expenditure; TEE/BW, total energy expenditure relative to body weight; RMR, resting metabolic rate measured by indirect calorimetry; AEE, activity energy expenditure.

N.A. RMR data was not available for the individual.

Appendix II Ethics Approval

St. Luke's – Roosevelt Hospital Center

Institute for Health Sciences

Institutional Review Board

432 West 58th Street, Room 207, New York NY 10019

Telephone (212) 523-4368, 4370, 6296 Fax (212) 523-7442

MEMO

To: Marie-Pierre St-Onge, PhD

From: Airlie Cameron, M.D., MPH

Chair, Institutional Review Board

Date: April 6, 2011

Re: Approval of Clarifications/Revisions for Initial Review of Full Board Reviews

IRB# 07-177

Title: Sleep Deprivation and Energy Balance [ICF Version Date Unspecified]

I am pleased to inform you that the Institutional Review Board has approved your submitted revisions and clarifications to the above cited research proposal. You may now begin the proposed research. Please note that for all sponsored research approval by the Grants Office must be obtained in addition to IRB approval prior to starting the research. The date of IRB approval has been stamped on your consent form.

Additionally, the Privacy Board has reviewed and approved the Research Authorization forms to be given to participants enrolled in the above-cited research project. Your stamped IRB/Privacy Board approved Authorization and Informed Consent Forms are enclosed.

Further changes in the protocol or informed consent may not be made without IRB review and approval. The only exception would be if these changes were necessary to eliminate apparent immediate hazards to the human subject. Any serious unanticipated adverse events or unexpected reactions, including death, loss of limb, need for operation, etc., should be reported by the Principal

Investigator in writing to the IRB within 48 hours of occurrence or receipt of report of occurrence.

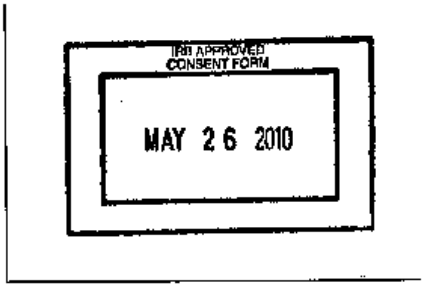
Your study will be due for continuing review by 3/18/2009. You will receive a notice of reminder one-month prior to that time.

FDA regulations require that you notify the IRB when your study is completed.

All correspondence concerning this matter should be submitted to the IRB Office, Antenucci 207. If you should have any questions, please contact the IRB Coordinator at 523-4368, 4370 or 6496.

Appendix III Study Forms and Questionnaires

Consent Form



Beth Israel Medical Center ☐

St. Luke's-Roosevelt Hospital Center ☐

CONSENT FOR PARTICIPATION IN RESEARCH

_____ Dr. Marie-Pierre St-Onge. Ph.D
Print name of subject Principal Investigator

Sleep deprivation and energy balance
Title of Project

IRB # 07-177

Attached to this form is a full description of the study in which we are asking you to participate. The description tells you about the reason for the study; the procedures, interviews and drugs or devices which may be involved; the duration of the study; and any risks or benefits to you. The description also gives you information about other medical treatments you may receive if you do not want to participate in this study.
If you have questions concerning this research project or your rights as a research subject, or if you have a research-related injury, you may telephone:

Patient Representative at: (212) 523-3700 Principal Investigator at: (212) 523-3564

CONSENT TO PARTICIPATE -- ADULT

I have read the attached study description. The purpose of the study, the risks of the study and what it means to participate in the study have all been explained to me, and my questions have been answered. I agree to participate in the study and agree to take all of the tests or procedures mentioned in the study description. If I am injured in the study, I understand only immediate essential medical treatment will be provided free of charge. I understand that participating in the study is voluntary, that I can decline to participate, and that I can stop participating at any time. I also understand that my decision to participate in or to withdraw from the study will not affect the health care I receive, now or in the future. I have been told that records of this investigation will be kept confidential to the extent permitted by law but are subject to inspection by the U.S. Food and Drug Administration and study sponsors.

_____ _____ _____ _____
signature of subject date signature of witness date

_____ _____
signature of authorized representative date relationship to subject

May only be used to enroll subjects until:

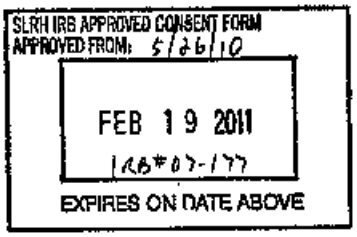
FEB 19 2011

I, _____, have clearly and fully explained to the above subject (or person giving consent) the nature, requirements and risks of the study.

_____ _____
Signature of researcher date

DISTRIBUTION:
Original to Research Records, copies for subject (or person giving permission), investigator, and Hospital Chart and Pharmacy where appropriate.

REV 05/07/10



Informed Consent

Protocol Name
Sleep deprivation and energy balance

Principal Investigator
Marie-Pierre St-Onge, Ph.D.

Sponsored by: National Institutes of Health

Please ask Dr. St-Onge or the Study Coordinator to explain anything in this consent form that you do not understand.

Description of study
You are being asked to join this research study which will examine the effect of sleep on the amount of calories that you burn and eat, and hormones that control food intake and the amount of calories that you burn. There is some research that suggests that there is a link between sleep duration and body weight. This study will investigate how sleep duration may have an impact on body weight. This study is not a weight loss study, nor do we assess your body composition. We simply want to understand how sleep can come to affect body weight. You will be one of approximately 29 other men and women to enroll in this study at St. Luke's Hospital. Clinilabs is the main site for this study but you will have some tests done at St. Luke's Hospital and Columbia University.

Procedures
The study will be conducted at Clinilabs (423 W55th Street, New York). Before being accepted into the study, you will be asked to wear an Actigraph monitor for a period of 2 weeks to monitor your sleep patterns. The Actigraph is a small device that you wear on your wrist, about the size of a large watch. You will also keep a sleep diary to record your bedtimes and wakeup times and a 3-day food diary to record all foods that you eat over a 3-day period. If you are eligible based on these measures, you will then undergo a drug screen and a pregnancy test (for women). You will be screened for anemia and will not be eligible to participate if your hematocrit (index of red blood cell count in the blood) is too low. You will be weighed and will be asked to fill out several questionnaires which ask you about your sleeping habits, caffeine intake, and the presence of metabolic disorders such as diabetes. You cannot give blood during the

study and for 3 months after the study and cannot travel across time zones during the study.

Once enrolled in the study you will be randomly assigned (like a flip of the coin) to one of 2 sleep sequences: short sleep followed by regular sleep or regular sleep followed by short sleep. During the short sleep period, you will be allowed to be in bed between 1 and 5 am. During the regular sleep period, you will be allowed to be in bed between 10 pm and 7 am. Each sleep period will last 5 nights. During this time (6 full days for each phase), you must remain at Clinilabs and will not be permitted to leave the premises unless you are accompanied by study personnel.

Each 6-day period will follow the same sequence of events. For the duration of time that you are at Clinilabs, you will wear the Actigraph to assess your activity level. All of the procedures listed below will be done twice.

On the first morning, you will come to Clinilabs for admission. The admission process at Clinilabs requires that you change into scrubs and have your belongings checked. You will not be permitted to take cigarettes, drugs, alcohol, food, or other inappropriate materials (for example, pornography) with you. If such items are found, they will be kept in a locked cabinet and returned to you upon discharge. We will also conduct a drug screening and breathalyzer test to ensure that you are healthy. We will take a measurement of your weight and will take your blood pressure. Your blood pressure will be taken after you sit and rest for 5 minutes and will be taken twice. A nurse will then take a blood sample. You must be fasted (nothing to eat or drink except for water) for 12 hours before your admission appointment. After we take your blood sample, you will provide a saliva and a urine sample. We will then give you a dose of doubly-labeled water (DLW) to drink. DLW is very similar to regular water except that the hydrogen and oxygen molecules are heavier. These are called "isotopes" of hydrogen and oxygen and they occur naturally; part of the oxygen and hydrogen that you breathe have these isotopes. They are also eliminated from your body rapidly and by measuring their rate of disappearance in your urine, we can measure how many calories you burn during your regular day-to-day life. Saliva samples will be collected 3 times that morning (before and 3 and 4 hours after DLW intake) and a urine sample will be taken on the evening of day 6. Also, each night that you will be at Clinilabs, you will have electrodes placed on your head during the night (only for the hours during which you are scheduled to be in bed). This test, called polysomnography, will measure your sleep. If, during the first and second nights, we find that you have sleep disordered

breathing (AHI) or periodic leg movement disorder (PLMD), you will be dismissed from the study.

All of your food will be provided for the duration of each 6-day period. However, during the first 4 days, we will determine what and when you eat. Your meals will be fixed in type and quantity and in the time at which they will be served. You will receive breakfast at 8 am, lunch at 12 pm, a snack at 4 pm, and dinner at 7 pm. On days 5 and 6, you will be given a choice of foods. You can eat as much or as little of any of foods that you select and you will have access to a snack box at all times. During these days, we will measure all of the foods that you consume. You cannot bring in any food from outside Clinilabs. The foods you eat must be provided by study personnel.

Every morning while you are at Clinilabs, you will have a fasting blood sample taken and your body weight, heart rate, and blood pressure will be measured. Also, on day 4, the nurse will insert a catheter into an arm vein to allow for multiple blood sampling without needing to stick you every time. Blood samples will be taken very frequently (at least every 2 hours) over the next 24 hours. The total amount of blood taken during each 6-day period will not exceed 315 mL (about 1 and 1/4 cup). You might have a small bruise on your arm where the needle is inserted. The blood will be stored at the New York Obesity Research Center and will be analyzed to measure your cholesterol, blood sugar, insulin and hormones involved in the regulation of food intake. Your samples will only be analyzed for the purposes described in this consent form.

On the morning of day 5, you will be taken to St. Luke's Hospital to have your resting metabolic rate measured. This measurement requires that you rest for 30 minutes before the measurement and remain lying on your back for another 30 minute period while we place a ventilated canopy over your head. Tubes will send air inside the canopy and collect the air that you breathe out to determine how much oxygen you consume at rest. This will tell us how many calories you burn at rest.

On the morning of day 6, we will take you to the Functional Magnetic Resonance Imaging (fMRI) Laboratory at Columbia University to do a scan of your brain. fMRI measures your brain activity in response to various stimuli. In this study, the stimuli will be objects and food. You will be placed in the scanner and shown objects and food over 2 periods of 5.5 minutes. The entire scan will take approximately 45 minutes (including set-up time and image acquisition). During this time, it is very important that you remain attentive to the items that are shown to you and that you not move your

head. During the scan, you will press a button on a box for each food or object seen. You will push the button with your index finger when you see a food or object with which you have been in contact during the week before your admission at Clinilabs and push the button with your middle finger when you see a food or object with which you have not been in contact during the week before your admission at Clinilabs. You will also perform a Stroop test. For this test, you will be shown words in different colors. For example, words will include red, blue, green. These words will sometimes be presented in the color that they represent (red typed in red ink) or not (red typed in blue ink). You will be asked to press a button based on what the word says, regardless of its color. For example, you press the button for red when you read the word red.

fMRI is non-invasive and does not involve radiation. It measures brain activity by measuring blood flow in your brain. The scanner is very loud. You will be provided with earplugs to reduce the noise. While in the scanner, you may feel claustrophobic (fear of being in closed spaces). The scanner is open on both ends but if you feel upset or uncomfortable, you must tell the technician and you will be taken out of the scanner.

The fMRI scan will be reviewed for any abnormalities. You will be notified if any abnormality is detected.

There will be a 2-4 week period separating the 2 6-day periods. During the 2 weeks prior to the second 6-day study period, you will have to wear the Actigraph again. This is done to ensure that your sleep has returned to normal. If we find that you have not recovered from sleep deprivation (if this was your first period), then we will extend the time separating the 2 study periods. For women, the period of time between study periods will correspond to the length of the menstrual cycle so that each study period falls within the same phase of the menstrual cycle.

Throughout the study periods, you will be asked to fill out some questionnaires regarding your appetite level and sleepiness. These questionnaires will be given on days 4 and 6.

Storage of Specimens

My blood samples will be analyzed for hormone measurements related to appetite and energy balance. The Principal Investigator will keep any left over blood samples for future studies. Blood samples will be retained for a maximum of 10 years after the

publication of the main results. Blood samples will be stored at St. Luke's Hospital's New York Obesity Research Center and will be identified based on your subject study code (2-letter initials, 3-digit subject code, 1-letter period code). Your name, date of birth, or any other personal identifying information will not be on these samples. Genetic testing may be done on these blood samples and your samples may be shared with other investigators. These investigators will not be informed of your identity. You can not participate in this study if you do not agree to have your samples stored for future testing. You will not receive the results of future tests.

I agree to have my blood samples analyzed for genetic testing:

Yes ☐ _____ No ☐ _____

If you change your mind with regards to genetic testing, please do so by writing to:

Dr. Marie-Pierre St-Onge
1090 Amsterdam Avenue, suite 14D
New York, NY 10025
Fax: (212) 523-3571

Eligibility Requirements

In order to qualify for the study, you must be between 30 and 45 years of age, with a stable body weight for the past 3 months. Your body mass index (BMI, defined as weight in kilograms divided by your height in meters squared) must be between 22 and 25. You must be healthy, not taking any medications which would affect your body weight, and have no neurological, medical, or psychiatric disorders. You must also regularly sleep between 7 and 9 hours per night and not take any daytime naps. You may not participate in this study if you are diabetic, have any eating or sleep disorder, or have a history of alcohol and drug abuse. Also, you cannot participate if you plan to travel across time zones within 4 weeks of the study or during the study, if you are a shift worker, a smoker, or high caffeine drinker.

Summary of Procedure Risks

You will be sleepy during the short sleep period. However, study personnel will be there to keep you company and prevent you from falling asleep. You may have slight bruising at the place of venipuncture during the blood draws. You may also feel

claustrophobic in the scanner and be uncomfortable due to the loud noise. However, you will have earplugs, which will decrease the noise level, and you will be in contact with study personnel during the entire scanning period. If you are uncomfortable, you will be taken out of the scanner at your request. The MRI may uncover previously unknown or undiagnosed conditions. You will be informed of any abnormal findings. Any abnormal findings should be discussed with and treated by your regular doctor.

After the period of sleep deprivation, you should use caution in doing every day tasks as you will feel sleepy. The extent of sleepiness and the time to full recovery is different for everyone. In general, you should expect to feel sleepy and less attentive than usual for a period of 1 week. During this period, you may be more vulnerable to accidents and should not operate vehicles or other heavy equipment.

There is a slight chance that you may have an allergic reaction to the adhesives used to secure the catheter or electrodes for the polysomnography.

Injury

If you are injured as a result of participation in the study only immediate essential medical treatment will be provided free of charge. Please contact Dr. St-Onge at 212-523-3564 or 917-526-3276 to report any injury resulting from your participation in this study.

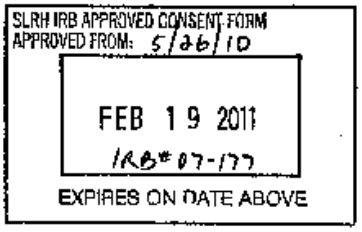
Benefits of Participation

The primary benefit to you as a result of your participation is to know the amount of calories that you burn at rest and how sleep can affect your appetite and the number of calories that you burn. You will be given the results of your individual tests as well as the average of all participants for each phase when the study is completed. Your participation in this study will make a big impact on the field of sleep and obesity and will allow the scientific community to have a better understanding of the impact of sleep on body weight control.

You can withdraw from the study at any time without jeopardizing your continued treatment at this institution. Any new information that may affect your willingness to continue your participation in this study will be brought to your attention.

Alternatives

You may choose not to participate in this study.



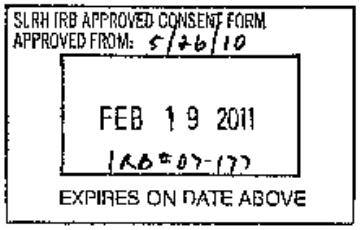
Voluntary Participation
Your participation in this study is entirely voluntary. Your refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. You may discontinue your participation at any time during the study without penalty or loss of benefits to which you are otherwise entitled.

Compensation
You will be compensated for your participation in this study. You will receive $750.00 at the completion of the first 6-day period and $1000.00 at the completion of the second 6-day period. If you complete both study periods, you will receive $1,750.00 to compensate for your time commitment in this study. Should you decide to withdraw from the study at any time before its completion, you will be compensated in a pro-rated amount as follows: Phase I, first 3 days, $40/day, completed 24-hour blood sampling, $80, completed fMRI $50, completed resting metabolic rate test $50; Phase II, first 3 days $40/day, completed 24-hour blood sampling, $150, completed fMRI $75, completed resting metabolic rate test $75. If you complete all of Phase I but not Phase II, you will be paid $500 for Phase I and any portion of Phase II completed as detailed above.

Duration of Study
Active treatment in this study is for a total of 12 days. However, you will be considered enrolled in this study for a period of 4-8 weeks (including time between active study periods).

Who to Call For Questions
If you have any questions about the study you may contact Marie-Pierre St-Onge, Ph.D. at 212 523-3564. If you have any questions about your rights as a patient you may contact the Patient Representative at 212 523-3700.

Confidentiality
If you consent to participate in this research, your personal information will be kept confidential and will not be released without your written permission, except as required by law. Your personal information may be shared, to the extent necessary, among the research staff, with the Institutional Review Board and research oversight staff, and/or with your treating physician or your other health care providers.



Your study information will also be sent to the Sponsor of this study (the National Institutes of Health) on study report forms. Your name will not be reported in any publication, only the data obtained as a result of your participation in this study will be made public. The sponsor of the study, and/or federal regulatory agencies, may inspect records identifying you as a subject in this investigation.

All data stored electronically will be kept in password-protected computers. In addition, all paper files will be stored in locked cabinets in the Principal Investigator's office. This office is kept locked at all times when no one is in the office. Only the Clinical Coordinators associated with this study will have access to those files.

Urine samples will be sent to the University of Manitoba, Winnipeg, Canada, for analyses. These samples will have no identifying information. Blood samples may be shared with other investigators. These samples will not have any identifying information.

We must provide your name and year of birth to Columbia University to schedule your fMRI scan. However, your actual scan will be initially stored under a code (3 letters of your last name and first letter of your first name and year of birth) and this code will be changed to your subject code for analyses.

If your scores on the depression questionnaire should be high enough to warrant medical follow up, we will forward your test results to your primary care physician for evaluation.

Costs
There are no costs to you for participating in this study.

If you fail to return the Actigraph at within 5 days of completion of your participation in this study or after your decision not to participate in this study or 5 days after your missed scheduled screening appointment, you will be charged $500 for replacement of the device.

You will be given a copy of this consent document.

Appendix III

Telephone Screening Script

"Thank you for your interest in this study. Before I ask you any questions to determine whether you are eligible to participate in this study, let me give you some information about this research project. If, after hearing about it you are still interested, I will ask you some questions about your health status. Your participation in this process is entirely voluntary and you may decide that you are not interested. In that case, I will not ask you any questions about your health. If, at the end of the health questionnaire you choose to not participate in the study, this information will be destroyed. Is that good with you?"

If no, thank the person and hang up. If yes, continue with the following:

"There is some research that suggests that sleep and body weight are related; short sleep being a risk factor for weight gain. We don't really know how sleep can affect weight so the purpose of this study is to examine the effects of sleep on food intake and the amount of calories that you burn. We also want to determine if sleep affects hormones that help you control your weight. In order to test this, we are looking for individuals who can participate in a 2-phase study: one phase with 4 hours of sleep per night for 6 nights and the other phase with 9 hours of sleep per night for 6 nights. For this study, you are required to stay at the research center and become inpatients for 2 periods of 6 consecutive days. Would you be willing to do this?"

If no, thank the person and hang up. If yes, continue with the following:

"Before I continue explaining the study, can I ask you a few questions to determine your potential eligibility?"

If no, thank the person and hang up. If yes, proceed with questions on Form 1 **(DO NOT ASK DATE OF BIRTH; only age is needed at this time)**. If one criterion is not met, thank the person and hang up. If all criteria are met, continue with the following:

"Each 6-day period will follow the same protocol. Basically, for the first 4 days, we will prepare all of your food for you and you will be required to eat all of the food that we give you at the times that we tell you. During the last 2 days, you will get to choose what, when, and how much you eat. We will weigh and measure the quantity of food and beverages that you consume. We will take fasting blood samples every day. On day 4, we will place a catheter in your arm and take frequent blood samples. On day 5, we will measure your energy expenditure and your brain activity in response to various food and object stimuli. Also, at the beginning of each phase, we will give you doubly-labeled water, a stable isotope of water that is naturally present in the atmosphere, to determine how many calories you burn over a 1-week period. The 2 study periods will differ

only in your bedtimes and wakeup times. During one period, you will go to bed at 1 am and wake up at 5 am and during the other period, you will go to bed at 10 pm and wake up at 7 am. Do you think you can comply with the demands of this protocol?

If no, thank the person and hang up. If yes, continue with the following:

"Let me ask you some further questions about your health to determine if you are eligible to participate in this study."

Proceed with questions on Form 2.

If, at any point during Form 2 the person is not eligible, thank him/her and hang up. If, after answering the above questions from the questionnaire the person is eligible, continue with the following:

"You may be eligible to participate in this study. Would you like to give me your name and phone number and we can schedule a time for an in-person screening and to review the consent form?"

Fill out **item #6** of Form 9. The person will be screened in person and will review the consent form in person at the Weight Control Unit. In person screening will cover Forms 3–9.

Appendix III

Basic Screening Form

SLEEP STUDY Subject ID: _____

SCREENING FORM Date: _____

Basic inclusion criteria Reviewer Initials: _____

Date of Birth:_____**Age**: _____**Sex**: ☐ Male ☐ Female

	e Age
	30-45

Height: _____ ft _____in

Weight: _____ lbs **Eligible Values**

Body Mass Index: _____ 22-25

Average hours of sleep/night: _____ hour/night 7-9

Smoker? ☐ Yes ☐ No No

Takes naps during day? ☐ Yes ☐ No No

Actigraphy monitoring:

Average recorded sleep time _____ 7-9

Average sleep time between 7 & 9 hours/night? ☐ Yes ☐ No Yes

At least 10 nights with >7 hours of sleep? ☐ Yes ☐ No Yes

Less than 4 nights with <6 hours of sleep? ☐ Yes ☐ No Yes

Based on the forms 1-7 and the Brief Symptoms Inventory questionnaire, is the subject eligible for inclusion in the study? ☐ Yes ☐ No

Comments: _____

Investigator Name: _____Date: _____

Appendix III

Subject Eligibility Form

SLEEP STUDY FORM SUBJECT ID: _____

SUBJECT ELIBILITY FORM SUBJECT INITIALS: _____

DATE: _____

REVIEWER INITITIALS: _____

SUBJECT ELIGIBILITY CRITERIA

Please mark (x) Yes or No for each criterion

Yes	No	INCLUSION CRITERIA
		1. Age 30-45 yrs;
		2. Male and non-pregnant, non-lactating female subjects
		3. Body mass index (BMI) 22-25 kg/m^2
		4. Weight stable (± 2.5 kg) for at least 3 mo prior to evaluation;
		5. If a woman of child-bearing potential, must be willing to adhere to an acceptable form of contraception;
		6. Non-smoker;
		7. Regularly sleeps 7-9 hours/night;
		8. Right-handed;
		9. If taking any form of medication, other than those listed in the exclusion criteria, must have been stable and remain on the same medication and medication dose throughout the study.
		10. Understands and is willing to sign informed consent; ***Date signed:*** ____/____/_____
		EXCLUSION CRITERIA
		1. Contraindication for MRI scanning;
		2. Diabetes, uncontrolled hypertension;
		3. Attempted to lose weight in past 3 months;
		4. Eating disorder;
		5. Stroke, seizure disorder, or other significant neurological disease;

		6. HIV positive by self-report
		7. Unstable or uncontrolled medical illness including active malignancies within past 5 yrs;
		8. Untreated or unstable hypothyroidism;
		9. Hyperthyroidism;
		10. A score on the Brief Psychiatric Inventory that exceeds the 90th percentile;
		11. Anemia;
		12. Subjects with psychoses, bipolar disorder, major depression, severe personality disorders, suicidal;
		13. Alcohol or substance abuse in the past 6 mo;
		14. Pregnant, planning pregnancy in the next 6 mo, or breast-feeding;
		15. Participating in a commercial diet or behavior modification program (e.g., Weight Watchers), or plans to participate;
		16. Shift worker, commercial long-distance driver, heavy equipment operator, history of drowsy driving;
		17. Takes naps regularly;
		18. Has traveled across time zones in the past 4 weeks or plans to during the weeks of the study;
		19. Excessive caffeine intake.
		Does subject have a history of any medical conditions not outlined above?
If Yes, give a brief description:		

If answers to all inclusion criteria are <u>YES</u> and all exclusion criteria are <u>NO</u>, then subject is eligible for study.

Appendix III

Medical Screening Form

SLEEP STUDY
SCREENING MEDICATIONS
SUBJECT ID: _____
SUBJECT INITIALS: _____
DATE: _____
REVIEWER INITIALS: _____

Is subject on any medication? ☐ **Yes** ☐ No

Medication # 01
Drug name: _____
Dose/unit: _____ Frequency: _____
Reason for use: _____
Date started: ___/___/_____ ☐ Ongoing

Medication # 02
Drug name: _____
Dose/unit: _____ Frequency: _____
Reason for use: _____
Date started: ___/___/_____ ☐ Ongoing

Medication # 03
Drug name: _____
Dose/unit: _____ Frequency: _____
Reason for use: _____
Date started: ___/___/_____ ☐ Ongoing

Medication # 04
Drug name: _____
Dose/unit: _____ Frequency: _____
Reason for use: _____
Date started: ___/___/_____ ☐ Ongoing

Medication # 05
Drug name: _____
Dose/unit: _____ Frequency: _____
Reason for use: _____
Date started: ___/___/_____ ☐ Ongoing

Medication # 06
Drug name: _____
Dose/unit: _____ Frequency: _____
Reason for use: _____
Date started: ___/___/_____ ☐ Ongoing

Medication # 07
Drug name: _____
Dose/unit: _____ Frequency: _____
Reason for use: _____
Date started: ___/___/_____ ☐ Ongoing

Medication # 08
Drug name: _____
Dose/unit: _____ Frequency: _____
Reason for use: _____
Date started: ___/___/_____ ☐ Ongoing

Medication # 09
Drug name: _____
Dose/unit: _____ Frequency: _____
Reason for use: _____
Date started: ___/___/_____ ☐ Ongoing

Appendix III

Pittsburgh Sleep Quality Index

SLEEP STUDY subject ID: _____

SCREENING FORM Date: _____

Basic inclusion criteria Reviewer Initials: _____

Pittsburgh Sleep Quality Index (PSQI)

Instructions: *The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions*

During the past month,

1. When have you usually gone to bed?_____

2. How long (in minutes) has it taken you to fall asleep each night?_____ (minutes)

3. When have you usually gotten up in the morning?_____

4. How many hours of actual sleep did you get that night? (This may be different from the number of hours you spend in bed) _____

5. During the past month, how often have you had trouble sleeping because you…	**Not during the past month (0)**	**Less than once a week (1)**	**Once or twice a week (2)**	**Three or more times a week (3)**
a. Cannot get to sleep within 30 minutes				
b. Wake up in the middle of the night or early morning				
c. Have to get up to use the bathroom				
d. Cannot breathe comfortably				
e. Cough or snore loudly				
f. Feel too cold				
g. Feel too hot				
h. Have bad dreams				
i. Have pain				

j. Other reasons(s), please describe, including how often you have had trouble sleep because of this reason(s):				
6. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?				
7. During the past month, how often have you had trouble Staying awake while driving, eating meals, or engaging in social activities?				
8. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?				
	Very good (0)	**Fairly good (1)**	**Fairly bad (2)**	**Very bad (3)**
9. During the past month, how would you rate your sleep quality overall?				

Component 1	#9 Score	C1_____
Component 2	#2 Score (≤15 min (0), 16-30 min (1), 31-60 min (2), >60 min (3) + #5a Score (if sum is equal 0=0; 1-2=1; 3-4=2; 5-6=3)	C2_____
Component 3	#4 Score (>7 (0), 6-7 (1), 5-6 (2), <5 (3)	C3_____
Component 4	(Total # of hours asleep) / (total # of hours in bed) x 100 >85%=0, 75%-84%=1; 65%-74%=2; <65%=3	C4_____
Component 5	# sum of scores 5b to 5j (0=0; 1-9=1; 10-18=2; 19-27=3	C5_____
Component 6	#6 Score	C6_____
Component 7	#7 Score = #8 score (0=0; 1-2=1; 3-4=2; 5-6=3)	C7_____

Add the seven components scores together _____ **Global PSQI Score**_____

Reprinted from Buysse DJ, Reynolds CF III, Monk TH, Berman SR & Kupfer DJ. The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. *Journal of Psychiatric Research* 1989; 28(2), 193-213

The Epworth Sleepiness Scale

SLEEP STUDY Subject ID: _____

SCREENING FORM Date: _____

Basic inclusion criteria Reviewer Initials: _____

The Epworth Sleepiness Scale (ESS)

Answers to the questions are rated from 0 to 3, with 0 meaning you would *never* doze or fall asleep in a given situation, and 3 meaning that there is a *very high likelihood* that you would fall asleep in that situation.

0 = would never doze **2 = moderate chance of dozing**

1 = slight chance of dozing **3 = high chance of dozing**

Situation	**Chance of dozing (0 to 3)**
Sitting and reading ……..	☐
Watching television……..	☐
Sitting inactive in a public place, for example, a theatre or meeting……	☐
As a passenger in a car for an hour without a break……..	☐
Lying down to rest in the afternoon……	☐
Sitting and talking to someone……	☐
Sitting quietly after lunch (when you've had no alcohol)……	☐
In a car, while stopped in traffic…….	☐
Your Total:	☐

Johns MW. A new method for measuring daytime sleepiness: the Epworth Sleepiness Scale. *Sleep* 1991; 14:540-545

Appendix III

Berlin Questionnaire

SLEEP STUDY Subject ID: _____

SCREENING FORM Date: _____

Basic inclusion criteria Reviewer Initials: _____

Berlin Questionnaire© 2000

Height: ____ Feet ____ Inches Weight: _____ kg/lbs. Age: _____ Gender: Male Female

CIRCLE THE ANSWER

Category 1

1. Do you snore?
   a. Yes
   b. No
   c. Don't know

IF YOU SNORE:

2. Your snoring is...
   a. slightly louder than breathing
   b. as loud as talking
   c. very loud. Can be heard in adjacent rooms

3. How often do you snore?
   a. Nearly every day
   b. 3-4 times a week
   c. 1-2 times a week
   d. 1-2 times a month
   e. Never or almost never

4. Has your snoring ever bothered other people?
   a. Yes
   b. No

5. Has anyone noticed that you quit breathing during your sleep?
   a. Nearly every day
   b. 3-4 times a week
   c. 1-2 times a week
   d. 1-2 times a month
   e. Never or nearly never

Category 2

6. How often do you feel tired or fatigued after your sleep?
   a. Nearly every day
   b. 3-4 times a week
   c. 1-2 times a week
   d. 1-2 times a month
   e. Never or nearly never

7. During your wake time, do you feel tired, or fatigued, or not up to par?
   a. Nearly every day
   b. 3-4 times a week
   c. 1-2 times a week
   d. 1-2 times a month
   e. Never or nearly never

8. Have you ever nodded off or fallen asleep while driving a vehicle?
   a. Yes
   b. No

IF YES:

9. How often does this occur?
   a. Nearly every day
   b. 3-4 times a week
   c. 1-2 times a week
   d. 1-2 times a month
   e. Never or nearly never

Appendix III: Berlin Questionnaire

SLEEP STUDY
SCREENING FORM
Basic inclusion criteria
Category 3

Subject ID: _____
Date: _____
Reviewer Initials: _____

10. Do you have high blood pressure?
    a. Yes
    b. No
    c. Don't know

Appendix III

Caffeine Consumption Questionnaire

SLEEP STUDY

SCREENING FORM

Basic inclusion criteria

Subject ID: _____
Date:
_____
Reviewer Initials:
_____

CAFFEINE CONSUMPTION QUESTIONNAIRE

	HOW OFTEN DO YOU DRINK THE FOLLOWING BEVERAGES?(MARK ONE)									**AMOUNT**			
	Never or less than once per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day	Medium serving size	Small	Medium	Large
Decaffeinated coffee (instant & brewed)	O	O	O	O	O	O	O	O	O	1 cup (8oz)	O	O	O
Instant coffee, not decaffeinated (including flavored types)	O	O	O	O	O	O	O	O	O	1 cup (8oz)	O	O	O
Brewed coffee, not decaffeinated	O	O	O	O	O	O	O	O	O	1 cup (8oz)	O	O	O
Decaffeinated espresso and espresso drinks (Latte, Mocha, Americano)	O	O	O	O	O	O	O	O	O	1 shot of espresso	O	O	O
Espresso and espresso drinks, not decaffeinated (Latte, Mocha, Americano)	O	O	O	O	O	O	O	O	O	1 shot of espresso	O	O	O
Herbal or decaffeinated tea (Instant, bottled, and brewed)	O	O	O	O	O	O	O	O	O	1 cup (8oz)	O	O	O
Green tea (Not decaffeinated-insant, bottled and brewed)	O	O	O	O	O	O	O	O	O	1 cup (8oz)	O	O	O
Black Tea such as Lipton or Earl Grey	O	O	O	O	O	O	O	O	O	1 cup (8oz)	O	O	O
Jolt, Surge, Mountain Dew, Red Bull and other highly caffeinated sodas	O	O	O	O	O	O	O	O	O	1 can (12oz)	O	O	O

Regular colas and root beer (with caffeine, not diet)	O	O	O	O	O	O	O	O	O	1 can (12oz)	O	O	O
Diet colas and diet root beer (with caffeine)	O	O	O	O	O	O	O	O	O	1 can (12oz)	O	O	O
Regular colas and root beer (caffeine free, not diet)	O	O	O	O	O	O	O	O	O	1 can (12oz)	O	O	O
Diet colas and diet root beer (caffeine free)	O	O	O	O	O	O	O	O	O	1 can (12oz)	O	O	O
Over the counter tablets (Excedrin, midol, no-doz, aspirin)	O	O	O	O	O	O	O	O	O	2 tablets	O	O	O
Over the counter syrups (cough, cold)	O	O	O	O	O	O	O	O	O	2 tbs.	O	O	O
Chocolate (milk, bar, baking, candy bar)	O	O	O	O	O	O	O	O	O	1 oz.	O	O	O

Questionnaire to Determine Morningness and Eveningness in Human Circadian Rhythms

SLEEP STUDY Subject ID: _____

SCREENING FORM Date: _____

Basic inclusion criteria Reviewer Initials: _____

Questionnaire to Determine Morningness and Eveningness in Human Circadian Rhythms

Instructions:

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

The Questionnaire

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

AM 5 6 7 8 9 10 11
12 Noon

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

AM 5 6 7 8 9 10 11
12 Noon

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

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

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

Not at all easy. …….. ☐
Not very easy …… ☐
Fairly easy ……. ☐
Very easy……..…….. ☐

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

Not at all alert. …….. ☐
Slightly alert ……. ☐
Fairly alert ……. ☐
Very alert……. …….. ☐

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

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

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

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

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

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

9. You have decided to engage in some physical exercise. A friend suggests that you do this one hour twice a week and the best time for him is between 7 am and 8 am. Bearing in mind nothing else but your own 'feeling best' rhythm, how do you think you would perform?

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

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

PM 8 9 10 11 12 midnight 1 AM 2 3

11. You wish to be at your peak performance for a test, which you know is going to be mentally exhausting and lasting for two hours. You are entirely free to plan your day. Considering your own 'feeling best' rhythm,' which one of the 4 testing times would you choose?

08:00 am – 10:00 am ……..... ☐
11:00 am – 01:00 pm……. ☐
03:00 pm – 05:00 pm …… ☐
07:00 pm – 09:00 pm …… ☐

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

Not at all tired. …….. ☐
Slightly tired ……. ☐
Fairly tired ……. ☐
Very tired ……..…….. ☐

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

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

14. One night you have to remain awake between 4:00 am and 6:00 am in order to carry out a night watch. You have no commitments the next day. Which ONE of the following alternatives are you most likely to experience?

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

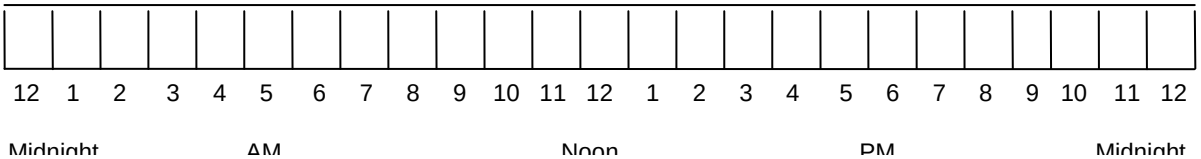
15. You have to do two hours of hard physical work. You are entirely free to plan your day. Considering only your own 'feeling best' rhythm, which ONE of the following times would you choose?

08:00 am – 10:00 am ……..... ☐
11:00 am – 01:00 pm……. ☐
03:00 pm – 05:00 pm …… ☐
07:00 pm – 09:00 pm ……. ☐

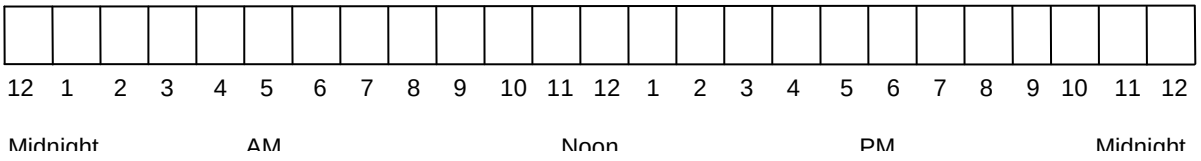
16. You have decided to engage in hard physical exercise. A friend suggests that you do this for one hour twice a week and the best time for him is between 10:00 pm and 11:00 pm. Bearing in mind nothing else but your own 'feeling best' rhythm, how well do you think you would perform?

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

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



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



19. One hears about 'morning' and 'evening' types of people. Which ONE of these types do you consider yourself to be?

Definitely a morning type..... ☐
Rather more a morning type than an evening type ..... ☐
Rather more an evening type than a morning type …… ☐
Definitely an evening type…… ☐

Appendix III

Patient Demographic Form

1. What is your race?

- ☐ White, not Hispanic
- ☐ Black, not Hispanic
- ☐ Hispanic
- ☐ American Indian
- ☐ Asian or Pacific Islander
- ☐ Other _____

2. What is your highest level of Education completed?

- ☐ Eighth Grade or less
- ☐ Some High School
- ☐ High School graduate
- ☐ Some College
- ☐ College graduate
- ☐ Graduate or Professional degree

3. What is your current employment status?

- ☐ Working, full-time
- ☐ Working, part-time
- ☐ Unemployed
- ☐ Retired
- ☐ Other _____

5. Are you currently, or have you been in the last 2 years, a St. Luke's/Roosevelt Hospital Center employee? ☐ Yes ☐ No

6. Personal Information:
   Name: _____ Address: _____
   Home Phone: ( ) _____ Alternate Phone: ( ) _____

7. **Emergency Contact**: Please list below a family member or friend whom we should contact in case of an emergency:
   Name: _____ Relationship: _____
   Address: _____
   Home Phone: ( ) _____ Work Phone: ( ) _____

Appendix III

Pre-Study Form

SLEEP STUDY
SCREENING FORM
Pre-study tests

Subject ID: _____
Date: _____
Reviewer Initials: _____

Eligible Values

PHASE 1

Actigraphy monitoring:

Average recorded sleep time _____ 7-9

Average sleep time between 7 & 9 hours/night? □ Yes □ No Yes

At least 10 nights with >7 hours of sleep? □ Yes □ No Yes

Less than 4 nights with <6 hours of sleep? □ Yes □ No Yes

Hematocrit

_____ Male

Female

PHASE 2

Actigraphy monitoring:

Average recorded sleep time _____ 7-9

Average sleep time between 7 & 9 hours/night? □ Yes □ No Yes

At least 10 nights with >7 hours of sleep? □ Yes □ No Yes

Less than 4 nights with <6 hours of sleep? □ Yes □ No Yes

Hematocrit

_____ Male

Female

Based on the forms 1-7 and the Brief Symptoms Inventory questionnaire, is the subject eligible for inclusion in the study? □ Yes □ No

Comments: _____

Investigator Name: _____ **Date:** _____

Appendix III

Health History Questionnaire

Original Date:
Reviewed by:

HEALTH HISTORY QUESTIONNAIRE

All questions contained in this questionnaire are strictly confidential and will become part of your research record.

Name *(Last, First, M.I.):* ☐ M ☐ F	**DOB:**
Marital status: ☐ Single ☐ Partnered ☐ Married ☐ Separated ☐ Divorced ☐ Widowed	
Previous or referring doctor:	**Person obtaining information:**

PERSONAL HEALTH HISTORY

Childhood illness: ☐ Measles ☐ Mumps ☐ Rubella ☐ Chickenpox ☐ Rheumatic Fever ☐ Polio		
Immunizations and dates:	☐ Tetanus	☐ Pneumonia
	☐ Hepatitis	☐ Chickenpox
	☐ Influenza	☐ MMR *Measles, Mumps, Rubella*

List any medical problems that doctors have diagnosed

Surgeries

Year	Reason	Hospital

Other hospitalizations

Year	Reason	Hospital

Appendix III: Health history questionnaire

Have you ever had a blood transfusion?	☐ Yes	☐ No

List your prescribed drugs and over-the-counter drugs, such as vitamins and inhalers		
Name the Drug	Strength	Frequency Taken

Allergies to medications	
Name the Drug	Reaction You Had

HEALTH HABITS AND PERSONAL SAFETY						
ALL QUESTIONS CONTAINED IN THIS QUESTIONNAIRE ARE OPTIONAL AND WILL BE KEPT STRICTLY CONFIDENTIAL.						
Exercise	☐ Sedentary (No exercise)					
	☐ Mild exercise (i.e., climb stairs, walk 3 blocks, golf)					
	☐ Occasional vigorous exercise (i.e., work or recreation, less than 4x/week for 30 min.)					
	☐ Regular vigorous exercise (i.e., work or recreation 4x/week for 30 minutes)					
Diet	Are you dieting?				☐ Yes	☐ No
	If yes, are you on a physician prescribed medical diet?				☐ Yes	☐ No
	Are you on a vegetarian diet?				☐ Yes	☐ No
	# of meals you eat in an average day?					
Caffeine	☐ None	☐ Coffee	☐ Tea	☐ Cola		
	# of cups/cans per day?					

Appendix III: Health history questionnaire

Alcohol	Do you drink alcohol?	☐ Yes	☐ No
	If yes, what kind?		
	How many drinks per week?		
	Are you concerned about the amount you drink?	☐ Yes	☐ No
	Have you considered stopping?	☐ Yes	☐ No
	Have you ever experienced blackouts?	☐ Yes	☐ No
	Are you prone to "binge" drinking?	☐ Yes	☐ No
	Do you drive after drinking?	☐ Yes	☐ No
Tobacco	Do you use tobacco?	☐ Yes	☐ No
	☐ Cigarettes – pks./day	☐ Chew - #/day	☐ Pipe - #/day	☐ Cigars - #/day
	☐ # of years	☐ Or year quit	
Drugs	Do you currently use recreational or street drugs?	☐ Yes	☐ No
	Have you ever given yourself street drugs with a needle?	☐ Yes	☐ No
Sex	Are you sexually active?	☐ Yes	☐ No
	If yes, are you trying for a pregnancy?	☐ Yes	☐ No
	If not trying for a pregnancy list contraceptive or barrier method used:		
Personal Safety	Do you live alone?	☐ Yes	☐ No
	Do you have frequent falls?	☐ Yes	☐ No
	Do you have vision or hearing loss?	☐ Yes	☐ No

HEALTH CONDITIONS

	PRESENCE	COMMENTS		PRESENCE	COMMENTS
Diabetes	☐ Y ☐ N		**Leg Swelling Leg Ulcers**	☐ Y ☐ N	
Hypertension	☐ Y ☐ N		**Venous Disease/DVT**	☐ Y ☐ N	
Heart Disease	☐ Y ☐ N		**Osteoarthritis**	☐ Y ☐ N	
Hyperlipidemia	☐ Y ☐ N		**Urinary Stress Incontinence**	☐ Y ☐ N	
Sleep Disorder	☐ Y ☐ N		**Menstrual/ Fertility Problems**	☐ Y ☐ N	
Asthma/Shortness of Breath	☐ Y ☐ N		**Skin Infections**	☐ Y ☐ N	
GE Reflux	☐ Y ☐ N		**Depression**	☐ Y ☐ N	
Gallstones	☐ Y ☐ N		**Other**		

Appendix III: Health history questionnaire

FAMILY HEALTH HISTORY

	AGE	SIGNIFICANT HEALTH PROBLEMS		AGE	SIGNIFICANT HEALTH PROBLEMS
Father			**Children**	☐ M ☐ F	
Mother				☐ M ☐ F	
Sibling	☐ M ☐ F			☐ M ☐ F	
	☐ M ☐ F			☐ M ☐ F	
	☐ M ☐ F		**Grandmother** *Maternal*		
	☐ M ☐ F		**Grandfather** *Maternal*		
	☐ M ☐ F		**Grandmother** *Paternal*		
	☐ M ☐ F		**Grandfather** *Paternal*		

MENTAL HEALTH

Is stress a major problem for you?	☐ Yes	☐ No
Do you feel depressed?	☐ Yes	☐ No
Do you panic when stressed?	☐ Yes	☐ No
Do you have problems with eating or your appetite?	☐ Yes	☐ No
Do you cry frequently?	☐ Yes	☐ No
Have you ever attempted suicide?	☐ Yes	☐ No
Have you ever seriously thought about hurting yourself?	☐ Yes	☐ No
Do you have trouble sleeping?	☐ Yes	☐ No
Have you ever been to a counselor?	☐ Yes	☐ No

WOMEN ONLY

Age at onset of menstruation:		
Date of last menstruation:		
Period every days		
Heavy periods, irregularity, spotting, pain, or discharge?	☐ Yes	☐ No
Number of pregnancies Number of live births		
Are you pregnant or breastfeeding?	☐ Yes	☐ No
Have you had a D&C, hysterectomy, or Cesarean?	☐ Yes	☐ No
Any urinary tract, bladder, or kidney infections within the last year?	☐ Yes	☐ No
Any blood in your urine?	☐ Yes	☐ No
Any problems with control of urination?	☐ Yes	☐ No
Any hot flashes or sweating at night?	☐ Yes	☐ No
Do you have menstrual tension, pain, bloating, irritability, or other symptoms at or around time of period?	☐ Yes	☐ No
Experienced any recent breast tenderness, lumps, or nipple discharge?	☐ Yes	☐ No
Date of last pap and rectal exam?		

MEN ONLY		
Do you usually get up to urinate during the night?	☐ Yes	☐ No
If yes, # of times		
Do you feel pain or burning with urination?	☐ Yes	☐ No
Any blood in your urine?	☐ Yes	☐ No
Do you feel burning discharge from penis?	☐ Yes	☐ No
Has the force of your urination decreased?	☐ Yes	☐ No
Have you had any kidney, bladder, or prostate infections within the last 12 months?	☐ Yes	☐ No
Do you have any problems emptying your bladder completely?	☐ Yes	☐ No
Any difficulty with erection or ejaculation?	☐ Yes	☐ No
Any testicle pain or swelling?	☐ Yes	☐ No
Date of last prostate and rectal exam?		

OTHER PROBLEMS
Check if you have, or have had, any symptoms in the following areas to a significant degree and briefly explain.

☐ Skin	☐ Chest/Heart	☐ Recent changes in:
☐ Head/Neck	☐ Back	☐ Weight
☐ Ears	☐ Intestinal	☐ Energy level
☐ Nose	☐ Bladder	☐ Ability to sleep
☐ Throat	☐ Bowel	☐ Anemia
☐ Lungs	☐ Circulation	☐ Other pain/discomfort:

Appendix III

Stanford Sleepiness Scale

SLEEP STUDY Subject ID: _____

Phase: 1 / 2 Date: _____

Sleep Allowance: 4 hours / 9 hours Reviewer Initials: _____

STANFORD SLEEPINESS SCALE (SSS)

AN INTROSPECTIVE MEASURE OF SLEEPINESS

Degree of Sleepiness	**Scale Rating**
Feeling active, vital, alert, or wide awake	1
Functioning at high levels, but not at peak; able to concentrate	2
Awake, but relaxed; responsive but not fully alert	3
Somewhat foggy, let down	4
Foggy; losing interest in remaining awake; slowed down	5
Sleepy, woozy, fighting sleep; prefer to lie down	6
No longer fighting sleep, sleep onset soon; having dream-like thoughts	7
Asleep	X

Appendix III: Stanford sleepiness scale

Please place an X in the appropriate column to rate your degree of sleepiness at each time. Use the ratings description above to guide you.

DAY 4								
TIME	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**X**
5 am								
6 am								
7 am								
8 am								
9 am								
10 am								
11 am								
12 pm								
1 pm								
2 pm								
3 pm								
4 pm								
5 pm								
6 pm								
7 pm								
8 pm								
9 pm								
10 pm								
11 pm								
12 am								
1 am								

Appendix III: Stanford sleepiness scale

Please place an X in the appropriate column to rate your degree of sleepiness at each time. Use the ratings description above to guide you.

DAY 5-6								
TIME	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**X**
8 pm								
9 pm								
10 pm								
11 pm								
12 am								
1 am								
5 am								
6 am								
7 am								
8 am								
9 am								
10 am								
11 am								
12 pm								
1 pm								
2 pm								
3 pm								
4 pm								
5 pm								
6 pm								
7 pm								

Appendix III

Hourly Appetite-Satiety Report

SLEEP STUDY SUBJECT ID: _____

APPETITE/SATIETY FORM SUBJECT INITIALS: _____

DAY 4

PHASE: 1 / 2 (circle)

SLEEP ALLOWANCE: 4 hours / 9 hours (circle)

To be completed by Clinical Coordinator

Please report your general feelings at this time on a 10-point scale, with 0 being not at all and 10 being very much so.

5 am	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
How hungry do you feel right now?											
How satisfied do you feel right now?											
How full do you feel right now?											
How much do you think you could eat right now?											
How energetic do you feel right now?											
How sluggish do you feel right now?											
How much would you like to eat something sweet right now?											
How much would you like to eat something salty right now?											
How much would you like to eat something savory right now?											
How much would you like to eat fruits & vegetables right now?											

NOTICE: There are 21 pages of the appetite/satiety report with same questions and scale standards, being given to participants to fill every hour from 5 am of d 4 to 1 am of d 5.

Appendix III

Significant but Not Serious Adverse Event Report Form

RECRUITMENT No _____
SUBJECT No _____
SUBJECT INITIALS: _____

Adverse Event Number _____
Did subject have any adverse events during the course of the study? ☐ Yes
If yes, complete section below. (× one)
☐ No

A Significant But Not Serious Adverse Event (AE) is defined as any unfavorable and unintended sign, including and marked hematological and other laboratory abnormality, symptom, or disease temporally associated with the use of a product, whether or not considered related to the product, including (but not limited to) those events resulting from use as stipulated in the protocol and that lead to an intervention by a healthy care professional who has examined the subject, including withdrawal of product, reduced product administration, or significant additional concomitant therapy.

(Do NOT include Serious Adverse Events).
Description _____

DATE EVENT DISCOVERED
___/___/_____

DATE OF ONSET
___/___/_____

DATE RESOLVED OR DURATION (If less than 24 hours) UNITS (× only one) OR ☐ ONGOING
___/___/_____ ___.___ ☐ hrs ☐ minutes

ACTION TAKEN (IF ANY) _____

SEVERITY (× only one)
☐ Mild -Transient and easily tolerated
☐ Moderate -Results in a modification or interruption of the subject's usual activities or care and may require discontinuation of the study product
☐ Severe -Results in considerable interference with the subject's usual activities or care and may require discontinuation of study product.

PRODUCT RELATED? (Investigator's Opinion) (× only one)

☐ Probable -An AE has a strong temporal relationship to study product or recurs on re-challenge and another etiology is unlikely or significantly less likely.
☐ Possible -An AE has a strong temporal relationship to study product and an alternative etiology is equally or less likely compared to the potential relationship to study product.
☐ Probably Not -An AE has little or no temporal relationship to the study product and/or a more likely alternative etiology exists.
☐ Not Related -An AE is due to an underlying or concurrent illness or effect of another product and is not related to the study product.

Appendix III

Deviation from Protocol

RECRUITMENT No _____
SUBJECT No _____
SUBJECT INITIALS _____

Describe any deviations from the protocol.

Were there any deviations from the protocol? ☐ Yes
If Yes, complete section below (× only one)
☐ No

Date of Deviation
___/___/_____
Month Day Year

Description

Deviation Type (× only one)

☐ Selection/enrollment
☐ Food consumption
☐ Randomization
☐ Study procedure
☐ Sleep duration

Deviation Type (× only one)

☐ Selection/enrollment
☐ Food consumption
☐ Randomization
☐ Study procedure
☐ Sleep duration

Deviation Type (× only one)

☐ Selection/enrollment
☐ Food consumption
☐ Randomization
☐ Study procedure
☐ Sleep duration

Appendix III

Exit Form

RECRUITMENT No _____
SUBJECT No _____
SUBJECT INITIALS _____

Date Subject Started Study:

____/___/_____
Month Day Year

Date Subject Completed or Dropped Study:

____/___/_____
Month Day Year

Reason Subject Exited From Study (✖ only one)

- ☐ Subject completed the study according to protocol
- ☐ Subject voluntarily dropped out prior to completion of study
- ☐ Subject never met entry criteria. Explain: _____
- ☐ Subject did not comply with the protocol. Explain: _____
- ☐ Subject experienced an adverse event necessitating study exit. Please record on Form 12.
- ☐ Subject was dropped by investigator. Explain: _____
- ☐ Subject expired. Date ___/___/____ Month Day Year
- ☐ Other. Explain: _____

Appendix III

Flyer

Are you a good sleeper?

If you are:

- **30-45 y**
- **Normal weight**
- **Non-diabetic**
- **Healthy**
- **Weight stable**

And sleep 7-9 hours/night, then you may qualify to participate in our Sleep Study!

If you are interested, please call 212-523-4603 to see if you qualify!

Sleep study 212-523-4603	Sleep study 212-523-4603	Sleep study 212-523-4603	Sleep study 212-523-4603	Sleep study 212-523-4603	Sleep study 212-523-4603	Sleep study 212-523-4603	Sleep study 212-523-4603	Sleep study 212-523-4603	Sleep study 212-523-4603	Sleep study 212-523-4603	Sleep study 212-523-4603	Sleep study 212-523-4603	Sleep study 212-523-4603	Sleep study 212-523-4603	Sleep study 212-523-4603		