Influence of Nocturnal Light Exposure on the Impairment of Glucose Tolerance Induced by Chronic Sleep Restriction

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I. BACKGROUND AND SIGNIFICANCE

This project is designed to test for the first time whether glucose metabolism is differentially impaired by sleep restriction with and without additional exposure to artificial light at night (ALAN). Laboratory studies have shown that sleep restriction to 4-6h per night for durations varying from one to 14 days reduces glucose tolerance in otherwise healthy adults [10-23,32,33], but the mechanisms by which insufficient sleep impairs glucose metabolism are still unknown. Current theories are based on the premise that the adverse metabolic consequences are caused by reduction in the duration of sleep *per se* [34]. However, sleep curtailment is typically accompanied by longer exposure to artificial light at night (ALAN), which is an environmental endocrine disrupter that profoundly disrupts circadian rhythms [24-27,35-37].

We have previously reported that acute circadian misalignment induced hyperglycemia comparable to pre-diabetic states in a third of otherwise healthy participants [55]. Since then, we have shown that even when the circadian phase of participants was realigned, prior exposure to 2 ½ weeks of chronic sleep restriction combined with a history of recurrent circadian disruption induced even more deleterious effects on glucose metabolism [32], in which pancreatic beta cells failed to respond adequately to increased glucose levels. Moreover, both night and rotating shift work (which induce circadian disruption) are associated with increased risk for metabolic problems (81)(82)(83)(84)(85)(86)(87). Night shifts can lead to acute increases in glucose and insulin levels (88), although some studies report reduced insulin release in response to meals consumed during the night (89-91). Given that circadian disruption has been shown to independently adversely affect metabolism, and exposure to ALAN adversely impacts metabolism in animals [38], it is important to understand the extent to which circadian disruption contributes to the observed impact of sleep curtailment on metabolism[35]. **No previous studies of the metabolic impact of sleep restriction in humans have controlled for this additional exposure to ALAN, thus confounding the effects of sleep restriction with the effects of circadian disruption caused by extended exposure to ALAN.**

Seminal work by Spiegel and colleagues [10] demonstrated that 6 nights of sleep restriction to 4h per night caused impairments in glucose tolerance in otherwise healthy young men. Subsequent sleep restriction studies have replicated and expanded on these initial results [11-23,32], establishing a clear association between sleep restriction and disrupted glucose tolerance. Many of these studies employed rigorous methods for ensuring the participants remained awake throughout the sleep restriction [13-15,19,22,32], for recording sleep [13-17,19,32], and for assessing metabolism [11,13,14,16,17,19,20,22,32], although this was not true for all. In fact, in some cases participants were only provided test meals (and otherwise had uncontrolled diets [21]), participants were allowed to leave the laboratory during the day (so that wakefulness and adherence to the study diet were not controlled [10,16,21]), baseline sleep was scheduled at fixed times that may have induced difficulty sleeping [14-16], or objective evidence for adherence to the extended wake episodes was not documented (potentially missing the presence of brief naps in sleep-restricted individuals).

Importantly, none of the previous sleep restriction studies have controlled for longer exposure to ALAN when imposing an extended wake duration [10,11,13,15,16,20-22]. Individuals with restricted sleep, whether in the laboratory (31, 130, 131) or in normal life (65), are exposed to extended photoperiod and to altered meal timing, which can differentially entrain central and peripheral circadian oscillators (98, 126). Retinal exposure to ordinary indoor light can impact the human circadian system (132-137), and during sleep restriction additional light exposure occurs in the late evening and/or early morning, times when the circadian system is most sensitive to light (137-142). This additional nighttime light exposure may cause phase shifts (143) and melatonin suppression (136, 137). However, most sleep restriction studies have not assessed or controlled for such circadian disruptions. There is also evidence from animal studies that, in addition to light exposure, sleep loss can have a direct impact on neuronal activity in the master circadian pacemaker (144), which in turn can influence peripheral clocks in metabolic organs through neuronal and hormonal signals [reviewed in (43, 101)]. Thus, given that exposure to ALAN is known to be an endocrine disruptor and to profoundly disrupt circadian rhythms, and endocrine and circadian disruption can adversely affect metabolism [28,29], it is probable that endocrine and circadian disruption caused by the extended exposure to ALAN may contribute to the adverse metabolic effects

induced by chronic sleep restriction. **We have therefore designed this study to be a direct comparison between sleep restriction with and without extended exposure to ALAN in order to distinguish the impact of sleep loss *per se* and sleep loss with extended photoperiod on glucose metabolism.**

Determining whether endocrine and circadian disruption induced by ALAN contributes to the mechanism by which chronic sleep deficiency leads to the development of obesity, insulin resistance, and type 2 diabetes is important given the widespread prevalence of sleep deficiency in Americans of all ages. **By clarifying a potential mechanism by which sleep restriction impairs glucose metabolism, our findings will lay the groundwork for the development of novel treatments and countermeasures to mitigate the adverse metabolic effects of chronic sleep restriction.** If extended ALAN-induced endocrine and circadian disruption is one of the mechanisms by which sleep restriction impairs glucose metabolism, targeted countermeasures could be developed (e.g. eyeglasses that minimize the endocrine and circadian disruptions induced by ALAN by reducing exposure to short-wavelength artificial light, use of lamps with a lower color temperature to minimize exposure to short-wavelength artificial light during episodes of extended wakefulness, software to reduce exposure to short-wavelength light from light-emitting screens, etc).

II. SPECIFIC AIMS

The goal of this project is to evaluate whether extended duration ALAN contributes to the adverse effects of sleep restriction on glucose metabolism. We plan to use a crossover design consisting of a 7-day baseline, 7-day sleep restriction (to 5h per night) with (LD 19:5) or without (LD 14:10) ALAN, 9-day washout, and another 7-day sleep restriction with or without ALAN (Figure 8). 20 healthy participants will be randomly assigned to receive ALAN in either the first or second exposure, balanced by age and sex. Specifically, we plan to:

1. Test the hypothesis that exposure to one week of sleep restriction with concurrent exposure to extended duration ALAN (LD 19:5) will induce greater impairment of insulin sensitivity than exposure to one week of sleep restriction without extended duration ALAN (LD 14:10). Insulin sensitivity (SI) will be assessed via an intravenous glucose tolerance test (ivGTT) before and after each week of exposure.
2. Test the hypothesis that exposure to one week of sleep restriction with concurrent exposure to extended duration ALAN (LD 19:5) will induce greater impairment of glucose tolerance than exposure to one week of sleep restriction without extended duration ALAN (LD 14:10). The 90-minute glucose area under the curve (AUC) will be assessed in response to a standardized meal both before and after each week of exposure.
3. Test the hypothesis that exposure to one week of sleep restriction with concurrent exposure to extended duration ALAN (LD 19:5) will reduce the duration of nocturnal melatonin secretion as compared to baseline more than exposure to one week of sleep restriction without extended duration ALAN (LD 14:10). The 24-h profile of melatonin secretion will be assessed during the lighting conditions that prevail throughout both sleep restriction conditions (i.e., 5h in darkness per night vs. 10h in darkness/near darkness per night). To determine the mechanism of this anticipated decrease in the duration of melatonin secretion (acute light-induced melatonin suppression vs. a reduction in the duration of the endogenous melatonin secretory profile), we will also measure the endogenous melatonin secretory profile on a ~24-hr recording on the day immediately after each week of intervention.
4. Exploratory Aim 4: Test the hypothesis that exposure to one week of sleep restriction with concurrent exposure to extended duration ALAN (LD 19:5) will lead to a greater reduction in the levels of long-chain highly unsaturated triglycerides than exposure to one week of sleep restriction without extended duration ALAN (LD 14:10). Plasma lipids will be assessed via mass spectrometry for a baseline and sleep-restricted sample from each participant in the above studies under ALAN and non-ALAN conditions. Because lower levels of long-chain highly unsaturated triglycerides are associated with a loss of insulin sensitivity [30], we will assess whether sleep restriction with ALAN leads to adverse changes in the levels of these triglyceride species that do not occur when sleep restriction is conducted without ALAN.
5. Exploratory Aim 5: test the hypothesis that exposure to one week of sleep restriction with concurrent exposure to extended duration ALAN (LD 19:5) will lead to a difference in the exhaled VOC profile compared with exposure to one week of sleep restriction without extended duration ALAN (LD 14:10).

6. Exploratory Aim 6: test the hypothesis that exposure to one week of sleep restriction with concurrent exposure to extended duration ALAN (LD 19:5) will lead to a change in the proteomic profile compared with exposure to one week of sleep restriction without extended duration ALAN (LD 14:10).
7. Exploratory Aim 7: test the hypothesis that the BodyTime assay will track changes in the melatonin profile and show a change in timing after exposure to one week of sleep restriction with concurrent exposure to extended duration ALAN (LD 19:5) compared to baseline.
8. Exploratory Aim 8: test the hypothesis that exposure to one week of sleep restriction will induce changes in objective human vocal parameters, both with and without extended duration ALAN.

III. SUBJECT SELECTION

A total of 20 healthy adults (20-40 years old) will be studied in this proposal. In order to obtain 20 people who meet all the study criteria and complete the 33-day inpatient study, we expect to enroll up to 100 people in the screening process for the study. Based on similar studies in the past, we anticipate that up to 25 individuals may be enrolled in the main study in order to have 20 complete the study. Approximately equal numbers of men and women will complete the study in each group.

Each potential participant will undergo an extensive screening procedure prior to participation in the study. The procedure begins with a telephone/online screening (to determine general medical suitability) and answering any questions potential participants may have about the study. If the original preliminary telephone/online screening questionnaire reveals no grounds for exclusion (see below), then potential participants are asked to come for an interview and physical screening. The physical screening includes a medical history, blood and urine tests, an ECG, and a physical examination by a physician or other qualified personnel (i.e physician assistant, certified nurse practitioner). Participants will also undergo psychological screening (see below), including questionnaires and a structured interview with a clinical psychologist. Finally, participants will be screened for sleep apnea and periodic limb movement disorder using a home sleep test.

We estimate that up to 10 individuals will inquire about the study for every 1 eventually studied, and that up to 5 individuals will begin the screening process for every 1 who successfully completes the screening and begins the study. We therefore plan to enroll up to 100 participants in the study in order to have 20 who pass all the screening and complete the 33-day study.

Inclusion/exclusion criteria.

(a) Medication/drug use. Potential participants must report no history of drug or alcohol dependency. Potential participants must report moderate or no use of caffeine, cigarettes and alcohol by history; be willing to abstain from caffeine, nicotine, alcohol and herbal medications for the duration of the ambulatory segment of the study; and will not be allowed to use these substances during the laboratory segment of the study. Potential participants who are regularly taking any prescription (with the exception for hormonal birth control; see Inclusion of Women and Minorities section) or over-the-counter medication will be excluded.

(b) Evaluation of medical suitability. Potential participants must be ambulatory and have no major visual or auditory handicaps. Participants must be free from any acute or debilitating medical conditions. Medical suitability will be determined by clinical history, physical examination, electrocardiogram, clinical biochemical screening tests of blood and urine.

Any participant with symptoms of acute or active illness (e.g., fever, leukocytosis, hypertension) will be excluded from study until those symptoms resolve. Given the wide range of illnesses that are encountered in medical practice, it would not be possible to provide a comprehensive list of each and every disease that could serve as grounds for exclusion for the participant. However, the following summarizes common medical conditions that will be exclusionary.

Diseases of the Cardiovascular System: hypertension (systolic blood pressure >130 or diastolic blood pressure >80), heart failure, cardiomyopathy, cor pulmonale, ischemic heart disease, valvular heart disease, history of heart transplantation, cardiac tumors, pericardial disease;

Disorders of the Respiratory System: asthma, cystic fibroses, chronic bronchitis, emphysema and airway obstruction, interstitial lung diseases, pulmonary hypertension, lung neoplasms, ARDS;

Disorders of the Kidney and Urinary Tract: acute or chronic renal failure, history of renal transplantation, tubulointerstitial diseases of the kidney, urinary tract obstruction, tumors of the urinary tract;

Infectious Diseases: infective endocarditis, HIV infection, sexually transmitted diseases [e.g., syphilis (including congenital syphilis and its sequelae), gonorrhea], osteomyelitis, brucellosis, tuberculosis, leptospirosis, Lyme disease, mononucleosis, parasitic infections such as toxoplasmosis, giardiasis, schistosomiasis, leishmaniasis;

Disorders of the Gastrointestinal System: esophagitis, peptic ulcer, gastritis, neoplasms of the esophagus, stomach or bowel, disorders of absorption, inflammatory bowel disease, diseases of the small and large intestine, acute appendicitis, hepatitis, cirrhosis or neoplasms of the liver, history of liver transplantation, diseases of the gallbladder and bile ducts, pancreatic disease, diabetes;

Disorders of the Immune System, Connective Tissue and Joints: AIDS, systemic lupus erythematosus, rheumatoid arthritis, scleroderma, ankylosing spondylitis, vasculitis, sarcoidosis, fibromyalgia;

Disorders of the Hematopoietic System: anemia, leukemia, myeloproliferative diseases, history of bone marrow transplantation;

Neoplastic Diseases: lymphoma, carcinoma, melanoma, or any other neoplastic diseases;

Endocrine and Metabolic Diseases: thyroid disease, Addison's Disease, Cushing's Syndrome, aldosteronism, hypoaldosteronism, pheochromocytoma, diabetes, disorders of sexual differentiation, disorders of neuroendocrine regulation, diseases of the anterior pituitary and hypothalamus, hemochromatosis porphyria, Wilson's Disease, glycogen storage diseases, diseases of the parathyroid gland, metabolic bone disease, disorders of phosphorus or magnesium metabolism, Paget's Disease;

Neurologic Disorders: epilepsy and disorders of consciousness, dementia, amnesic disorders, neoplastic diseases of the central nervous system, demyelinating diseases, Parkinson's Disease, muscular dystrophy, myasthenia gravis, periodic paralysis, dermatomyositis, polymyositis, infections of the nervous system, stroke, history of transient ischemic attacks, hydrocephalus, tumors of the pituitary gland, pinealoma, intervertebral disc disease, ataxia, Gilles de la Tourette Syndrome, Huntington's Disease, tardive dyskinesia, history of recurrent migraine or cluster headaches, neuromuscular disease.

Sleep Disorders: narcolepsy, sleep apnea, Periodic Limb Movement Disorder (PLMD), nocturnal paroxysmal dystonia, REM-sleep behavior disorder, nocturnal eneuresis.

(c) BMI. BMI inclusion criteria are 20 – 29.9 kg/m^2.

(d) Evaluation of psychiatric/psychological suitability. Under the supervision of Dr. [redacted] Ph.D., Associate Psychologist in the Division of Psychiatry at the Brigham and Women's Hospital/Harvard Medical School, each potential participant will be administered an MMPI II, Symptom Checklist 90R, Beck Depression Scale, and State Anxiety Scale. Dr. [redacted] is a clinical psychologist who has been carrying out psychological screening for participants in our research studies for more than three decades, and support for these pre-study evaluations is included in the proposed project budget. As the final part of the psychological screening evaluation of potential participants, Dr. [redacted] will conduct a structured interview (SCID-R) with each potential participant. In particular, Dr. [redacted] will evaluate potential participants for Axis II personality types that might interfere with compliance or with their personal ability to tolerate the conditions of the study. Dr. [redacted] will also evaluate whether potential participants demonstrate a full understanding of the requirements and demands of the study.

Individuals with a personal history of psychiatric illnesses or psychiatric disorders will be excluded, and those with a first-degree relative with psychiatric illness will also be excluded. That includes alcoholism, drug

dependency, major mood disorders such as major depression and manic depressive illness, schizophrenic disorders, anxiety disorders including panic disorder, generalized anxiety disorder, post-traumatic stress disorder, obsessive compulsive disorder, agoraphobia, claustrophobia, paranoid personality disorder, schizoid personality disorder, schizotypal personality disorder, borderline personality disorder, and antisocial personality disorder. Individuals who are unaware of specific psychiatric diagnoses but who have a history of having been treated with antidepressant, neuroleptic medications, or major tranquilizers will be excluded from study. However, a personal history of limited prior counseling or psychotherapy (e.g., for adjustment reactions) will not necessarily be exclusionary. Participants must demonstrate a full understanding of the requirements and demands of the study.

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Exclusionary Scores on Psychological Screening Tests

1) MMPI Scales:	
Depression scale	T > 70: excluded automatically
Psychopathic deviance, schizophrenia, hypomania scale	T > 75: excluded automatically*
L, F & K validity scales & other clinical scales	T > 80: excluded automatically
L, F & K validity scales & other clinical scales	T >70, but <80: held pending interview**
2) Beck Depression Scale	> 10: excluded automatically
3) State Anxiety Scale	> 40: excluded automatically
4) Symptom Checklist 90-R	
Depression scale Distress level	> 1.25: excluded automatically
Hostility scale	> 1: excluded automatically
Phobic anxiety	> 0.75: excluded automatically
Paranoid ideation	> 1.25: excluded automatically
Psychoticism	> 1: excluded automatically
Anxiety	> 1.25: excluded automatically

**Scores on all 3 of these scales are correlated with age; college and graduate students generally score higher on these scales, so we have adjusted our cutoff values accordingly.*

***Participants with these scores may be excluded; they will be referred to Dr.* [redacted] *for clinical interview, and inclusion/exclusion will depend on the results of that clinical interview.*

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(e) Work, travel, and sleep-wake history. Participants must report habitually sleeping between 7 and 9 hours per night. This will be verified using actigraphy and sleep diaries during the pre-study Ambulatory Baseline. Potential participants will complete the Pittsburgh Sleep Quality Index and the Epworth Sleepiness Scale prior to study, and individuals with evidence of significant sleep disruption (PSQI >5 or ESS >10) will be excluded. Individuals with a prior diagnosis of a sleep disorder, and those regularly taking prescription or over-the-counter sleep aids will be excluded.

Potential participants must have no history of working on an irregular schedule that requires overnight, early morning (shift starts before 6am), and daytime work for the three years prior to study. They must not have traveled across more than two time zones during the month prior to the study. These criteria are designed to ensure that the individual is stably entrained to local environmental time and to their individual sleep-wake schedule.

For the two weeks prior to entering the laboratory for study, all participants will maintain a regular sleep-wake and light-dark schedule (± 30 min) of 9-h duration, centered at their habitual times, will record these times in a sleep diary, and will phone in twice per day to record their sleep and wake times. They will also undergo ambulatory monitoring of their activity and light exposure levels using a small wrist activity monitor during those same two weeks to document their sleep-wake behavior prior to entering the laboratory, and to ensure they enter the study in a well-rested condition.

Inclusion of racial/ethnic minorities. Our recruitment procedures provide all applicants with an equal opportunity to participate in our studies regardless of race, ethnicity, or national origin.

Gender. We will attempt to include equal numbers of women and men in the proposed study. However, pregnant subjects or those attempting to become pregnant will not be studied. Women with symptoms of pre-menstrual dysphoric disorder (PMDD) will be excluded, as will those who are breastfeeding. Women who have been pregnant or who have been breastfeeding within the past 6 months will be excluded.

Source of subjects and recruitment methods.
We will recruit participants from the local community by posting ads on the internet and posting flyers on local bulletin boards, methods we find to be cost-effective for this target population. We will also use two methods within Partners Health Care, Research Patient Data Registry (RPDR)and Rally RPDR is a searchable database maintained by MGB hospital systems to provide patients that meet user-defined characteristics and criteria such as diagnoses, procedures, medications, and/ or laboratory results. We will also recruit previous study participants from a database of former participants who have given permission to be notified about future studies that they might be eligible for. Research Invitations will be used for direct recruitment of any eligible subjects who have not opted out of receiving Research Invitations and patients who opted out will be filtered out of the recruitment list and they will not be sent the Research Invitation. The PI will conduct ongoing monitoring of patient responses to ensure that our selection criteria are identifying the right patients. Complaints about this method of recruitment will be submitted to the IRB as an Other Event. The identity of potential volunteers is maintained until they choose to contact the Investigators. Rally is the Partners clinical trials website (clinicaltrials.partners.org) where Investigators can post study advertisements and interested community members can view all studies that match their age, sex, and other criteria. We will also recruit previous study participants. Drs. Czeisler, Duffy, Scheer, and Williams each carry out clinical research on healthy adults, and participants are asked at the end of their study if they wish to be notified about future studies that they might be eligible for.

IV. SUBJECT ENROLLMENT

This study uses a within-subjects randomized crossover design to compare the effects of sleep restriction with and without ALAN on glucose metabolism. The order in which subjects undergo the two sleep interventions will be randomized.

Procedures for obtaining informed consent (including timing of consent process)
Written informed consent will be obtained from each subject in a 2-step process. A Screening Consent Form will be used to obtain consent for the screening procedures. This will be obtained by a Study Coordinator/Recruiter or post-doctoral research fellow at the start of the first screening visit. A Research Consent Form will be used to obtain consent for the research study itself. Consent for the screening procedures will be obtained by a trained Research Assistant. Consent for the research study procedures will be obtained by the PI, a Co-Investigator, or a Research Fellow at least 24h before the first study day. We plan to use a REDCap eConsent module and secure videoconferencing (MGB-approved and HIPPA-compliant Zoom) or to do the consent in person when public health guidelines allow.

Participants who complete the prescreening form have the option to consent to being contacted by text message for study visit reminders during the screening process only. The MGB recommended consent text disclosing security risks of text messaging is shown to participants at the end of the prescreening form, and participants have the option to opt in to receive text reminders. Text reminders are not used during the inpatient study, nor are they used after completion of the research study. Text messaging will be carried out using Twilio, a secure texting platform integrated with REDCap through the REDCap + Twilio Module.

V. STUDY PROCEDURES

The experimental protocol consists of 6 segments: ambulatory baseline; laboratory baseline assessment of glucose metabolism and circadian rhythms; first sleep intervention including re-assessment of glucose

metabolism and circadian rhythms; washout followed by second baseline assessment of glucose metabolism and circadian rhythms; second sleep intervention including re-assessment of glucose metabolism and circadian rhythms, and recovery. The ambulatory baseline is roughly 2 weeks, after which participants will be studied in the laboratory for a 33-day protocol.

During the **Ambulatory Baseline**, wrist activity and light levels will be recorded in participants at home for approximately 2 weeks prior to study admission. They will be asked to wear an activity monitor (MotionWatch 8 or similar, CamNTech, Inc.) on the wrist of the non-dominant hand, maintain a regular self-selected sleep-wake schedule with 9h time in bed each night, record this schedule in a sleep diary, and call in to our time-stamped call-in telephone system just prior to each bedtime and immediately after each wake time. These procedures will ensure the participants are stably entrained to their schedule and well-rested when the study begins. We may also ask them to log the time, content, and amount of food and drink consumed each day during this time in a food diary. This will inform us of the participant's usual diet and allow comparison with the laboratory diet.

Immediately after the ambulatory baseline, the participant will be admitted to the Center for Clinical Investigation (CCI) at BWH on Day 1. They will be scheduled to sleep for 10 hours overnight at the CCI (centered at their usual times), and their sleep will be recorded with standard polysomnography (PSG) on some or all of the nights. Upon awakening the next morning (Day 2), the participant will begin the first **Baseline Day**. They will be given isocaloric, nutrient-controlled meals (breakfast, lunch, dinner) and will be scheduled to sleep again for 10 hours overnight. This will continue for the remaining baseline days. Near the end of the first Baseline segment (approximately Day 5), they will have an IV catheter inserted into a forearm vein for blood sampling. After awakening the next morning (~Day 6) after an overnight fast, the participant will undergo an assessment of their resting metabolic rate via indirect calorimetry. This will be followed by a Mixed Meal Response at breakfast to evaluate their baseline glucose and insulin response to a meal (see below for details). Frequent blood samples for melatonin will also be drawn to assess the duration of melatonin secretion and melatonin levels during the metabolic assessments, and this sample collection will continue throughout the next three days (~Days 6-8). Upon awakening on Day 7 after an overnight fast, the participant will undergo an assessment of their resting metabolic rate via indirect calorimetry again, and this will be followed by a baseline insulin-modified IV glucose tolerance test (GTT) that will be carried out to assess insulin sensitivity (S_I), acute insulin response (AIRg), and glucose effectiveness (see below). The IVGTT will be scheduled to begin at the same time as the Mixed Meal Response on the previous day. Sample collection for the IVGTT will be completed by approximately 3 hours later (4 hours after wake time). Beginning in the early afternoon, ambient lighting will be lowered to <15 lux and ~hourly blood samples for melatonin endogenous circadian rhythm assessment will be drawn. Sleep will be scheduled at the same time as Nights 1-6, and blood sampling will continue throughout the night. Sample collection for the circadian rhythm of melatonin will be completed by approximately 10 hours after wake time, at which time the sampling IV will be removed and the lighting will be returned to normal.

The next 7 nights are the **First Sleep Intervention** and will depend on the randomization order. In the Sleep Restriction Group with Extended Duration Artificial Light At Night (ALAN) first, the sleep episodes will be shortened to 5 hours, centered at the same time as the baseline sleep (with bedtime 2.5 hours later and wake time 2.5 hours earlier). In the Sleep Restriction group without Extended Duration ALAN, the sleep episodes will be shortened to 5 hours as in the ALAN Condition (centered at the same time as the baseline sleep with bedtime 2.5 hours later and wake time 2.5 hours earlier), but the participant will remain sitting in bed in near darkness (< 1 lux) for the 2.5 hours before and after the 5-hour sleep episode, such that their exposure to room lighting and activity (14 hours/day) will remain similar to that in the Baseline condition. <u>The timing of meals in both</u> conditions throughout this <u>intervention segment will remain the same as on the Baseline days</u>.

Near the end of the first sleep intervention (~Day 14), each participant will undergo a re-assessment of their resting metabolic rate via indirect calorimetry at the same time as during Baseline. This will be followed by a Standardized Mixed Meal Response at breakfast to re-evaluate their glucose and insulin response to a meal, along with frequent blood samples to assess duration of melatonin secretion and melatonin levels during the metabolic assessments. This sample collection for melatonin will continue throughout the next three days (~Days 14-16). Near the end of the first sleep intervention (~Day 15), an ivGTT re-assessment will be carried out to assess insulin sensitivity (S_I), acute insulin response (AIRg), and glucose effectiveness. This will begin at the

same time as the Baseline ivGTT. Sample collection for the ivGTT will be completed by approximately 3 hours later. Beginning in the afternoon, ambient lighting will be lowered to <15 lux and frequent blood samples for re-assessment of melatonin and cortisol circadian rhythms will be drawn, continuing until the following afternoon .

For the **Washout**, sleep episodes will be scheduled to 10 hours each night at the same time of the Baseline sleep episodes, from Days 15-23. Upon awakening after an overnight fast toward the end of the Washout (~Day 23), the participant will undergo the second **Baseline** assessment of their resting metabolic rate, glucose and insulin responses, and melatonin rhythms. Standardized Mixed Meal Responses, ivGTTs, and melatonin sampling will be carried out on the next 3 days (~Days 23-25) as performed during the first Baseline assessment on Days 6-8. Following the washout, the next 7 nights are the **Second Sleep Intervention**, during which participants will undergo the sleep restriction condition (with or without ALAN) which they have not yet undergone during the first sleep intervention.

Upon awakening after an overnight fast at the end of the 7-day exposure (~Day 31), the participant will undergo another re-assessment of their resting metabolic rate, glucose and insulin responses, and melatonin rhythms. Standardized Mixed Meal Responses, ivGTTs, and melatonin sampling will be carried out on the next 3 days (~Days 31-33) as performed during the assessment following the first Sleep Intervention on Days 14-16. The final **Recovery** sleep episode on Night 32 will be scheduled for 10 hours at the same time of the Baseline sleep episodes. Blood sampling will be completed approximately 10 hours after waketime on Day 33, at which time the sampling IV will be removed and the participant will be discharged. At the times indicated above when blood sampling is scheduled, we may collect additional small amounts of blood to assay for proteins, lipids, transcripts, or metabolites.

How Biospecimens, Data, and/or Records, will be Obtained. As outlined above, activity/light data and diary recordings, as well as a blood sample for DNA extraction will be collected from participants during the pre-study screening period. In the laboratory study, PSG sleep and wake recordings, answers to written and computerized questionnaires, whole blood and saliva biospecimen will be collected. We may also collect breath samples and fecal samples during specific days of the study.

Ambulatory physiologic monitoring. Wrist activity and ambient light levels will be monitored in participants at home for approximately 2 weeks prior to study admission. They will be asked to wear an activity monitor (MotionWatch 8 or similar, CamNTech, Inc.) on the wrist of the non-dominant hand, maintain a regular self-selected sleep-wake schedule with 10 h time in bed each night, record this schedule in a sleep diary, and call in to our time-stamped call-in telephone system just prior to each bedtime and immediately after each wake time. These procedures will ensure the participants are stably entrained to their schedule and well-rested when the study begins. They will also log the time, content, and amount of food and drink consumed each day during this time in a food diary. This will inform us of the participant's usual diet and allow comparison with the laboratory diet.

Inpatient environment and conditions. Participants will live in environmentally-controlled private rooms in the Intensive Physiological Monitoring (IPM) Unit of the Brigham and Women's Hospital (BWH) Center for Clinical Investigation (CCI) throughout the study. The rooms are equipped with hand-held terminals for on-line event recording, a porthole to enable 24-h blood sample collection without sleep disturbance, a closed-circuit camera and a voice-activated microphone for continuous monitoring, and neurobehavioral performance testing computer stations. Technicians are present 24h/ day to carry out the protocol, monitor the data acquisition systems, collect biologic specimen, record PSG, and respond to participant's requests. Research nurses are present 24h/day to insert and maintain the intravenous blood sampling system and to monitor the participant's health and mood, and an attending physician visits the participant daily to monitor their health. Specially-trained nurses, PAs and NPs are available to carry out IVGTTs. A dedicated specimen processing staff are present 24h/day to ensure all specimen are processed according to the requirements for the specific analytes to be assayed. Written protocols and checklists are used to ensure uniformity in the execution of standard procedures and to foster intra-staff communications (e.g., at shift change). Our research group has studied participants using these conditions for more than 30 years, and the staff members of the Division and the BWH CCI carry out such studies in the IPM on a daily basis.

Wake episodes. During this condition, on most days the participants will be free to move about their study suite as desired, except that they are instructed not to lie down or nap. The participant's activity will be monitored for compliance by means of closed-circuit cameras, and during the intervention week a study team member will remain in the room with the participant to ensure they do not fall asleep accidentally. See Sleep restriction section below for further details.

Lighting. During the circadian rhythm sample collection segments on Days 7-8, 15-16, and 24-25, maximum ambient light during scheduled wake will be <15 lux when measured vertically at 72" [90 deg] and ~5 lux measured horizontally at a height of 54" [0 deg]; “dim lighting”). In the Sleep Restriction without Extended Duration ALAN condition, the participants will be in “near darkness” for the 2.5 hours before and after each scheduled 5-hour sleep episode. This lighting will approximately 3 lux maximal exposure, measured at 72" vertically or <1 lux measured at 54" horizontally). Throughout all other wake episodes “normal indoor lighting” will apply. Ambient lighting is switched off (0 lux) during all scheduled bed rest episodes.

Sleep and polysomnographic recording. Throughout all scheduled sleep opportunities, participants will be required to remain supine in bed in the dark. Sleep episodes will be polysomnograhically recorded on some or all nights to document the duration of sleep [108] using Vitaport-3 ambulatory digital sleep recorders (DSR) or similar devices. Study staff will apply surface electrodes to specific face and scalp locations allowing sufficient time for the participant to dress and get into bed, and for the technical staff to connect the DSR and carry out calibration procedures. EEG will also be recorded throughout most of the scheduled wake episodes to document wakefulness [107]. The DSR will be connected to the control room via a special extension cord that will enable staff in the control room to monitor the signals online.

Sleep restriction. Our laboratory has well-established procedures for ensuring participants in sleep loss protocols remain awake throughout the entirety of their scheduled wake episodes [13, 32, 106, 107]. Participants may be directly monitored in their study room during the sleep intervention segments to ensure they remain awake and adhere to the protocol. The staff members who carry out this monitoring are trained to directly observe the face and eyes of the participant and to engage them in conversation or other activity (such as playing a board game) if the participant is struggling to remain awake. In the Sleep Restriction without Extended Light condition, the participants will be asked to remain awake while sitting in bed in near-darkness for 2.5 hours before and after each 5-hour sleep episode during the week of sleep intervention. We have experience ensuring participants remain awake even in such challenging conditions. The waking EEG recordings are reviewed on a regular basis and feedback is provided to the study staff if brief sleep episodes are detected.

Diet. Appropriate dietary control is important for ensuring that any modulation of glucose metabolism and other metabolic measures during the study are due to sleep or light exposure and not dietary changes such as calories, sodium, or other parameters that have been shown to modulate glucose metabolism. The diet throughout the inpatient portion of the study will be designed to be isocaloric, and the Mifflin St. Jeor equation [96] with a 1.6 activity factor will be used to calculate the caloric need of each participant. The diet will have a predetermined macronutrient percentage (58-60% carbohydrates, 15-17% protein, and 25-27% fat) and a set micronutrient intake (calcium 800-1000 mg, potassium 100 mEq [+/-20%], and sodium 150 mEq [+/-20%]) for all participants. The timing and content (macronutrients, %daily calories) of meals will be the same each study day, and between participants. Participants will be recruited and screened with full knowledge of the dietary control aspect of the study.

Resting Energy Expenditure (REE). REE will be assessed by indirect calorimetry using expired gases toward the end of each baseline and toward the end of each sleep intervention segment. Assessments will begin after waketime while the participant is fasting. Participants will remain in bed in a semi-recumbent posture while the equipment is set up and calibrated. The recording will last for ~15-30 minutes, during which the participant will breathe through a disposable mouthpiece into validated and FDA-approved indirect calorimeter (VMAX29 Encore, Carefusion, or similar device). The calorimeter estimates resting energy expenditure (REE, in kcal/day) and RQ (a dimensionless number corresponding to the amount of expired VCO_2 per amount of oxygen

consumed (VO_2). Carbohydrate oxidation and lipid oxidation rates can also be calculated (as grams per minute [g/min] according to the formulae of Frayn [116], assuming negligible protein oxidation [103-105].

Blood sample collection. The BWH CCI has a system for collecting frequent blood samples from participants even while they are sleeping. This system uses an indwelling intravenous catheter placed in a forearm vein by a CCI nurse. The catheter is connected to a triple-stopcock manifold via an intravenous loop with a 12-foot small-lumen extension cable through which blood sampling can continue in the next room without disturbing the participant during sleep. Between samples, a solution of 0.45% saline with 5,000 IU/liter of heparin will be infused at a rate of 40 cc/hour to maintain patency. This blood sample collection system has been in use by our research group for more than 30 years [97]. CCI technical staff and nurses are trained in the collection of multiple, frequent blood samples using this system, and it is in routine use on the BWH IPM on a daily basis. We will use this system to draw the samples for baseline and end of intervention circadian rhythm assessments, to draw the fasted samples and frequent blood samples for the baseline and end of intervention Mixed Meal Responses and IVGTT samples, and to draw samples for exploratory -omics analyses.

Standardized Mixed Meal Response test. Post-prandial mixed meal metabolic responses will be assessed toward the end of each baseline and toward the end of each sleep intervention segment following the measurement of RMR/REE. This will be done after an overnight fast using a breakfast [32]. The meal will have the overall composition of the diet described above, but each participant will be served an identical breakfast at each test and will be required to eat all of the meal within 30 minutes. Blood samples (approximately 4mL) will be collected using the sample collection system described above, with careful attention to the timing of the start of the breakfast, and will include pre-meal baseline samples as well as post-prandial samples at ~10-min intervals for the first 90 minutes following the meal, then at ~30-min intervals for the next 90 minutes (total of 3 hours). The participant will remain sitting in bed until the final blood sample is drawn. The samples will be placed into collection tubes and centrifuged, then pipetted into small polystyrene tubes and stored at -80°C for later analysis of glucose, insulin, and other hormones using standard procedures and/or commercially available kits.

Hormonal data. The recording of the circadian rhythm of melatonin is essential, as it is considered a “gold standard” way to assess central circadian phase. The melatonin rhythm has been demonstrated to be the most precise and accurate marker of the human circadian pacemaker [98-100]. Samples of blood will be collected at approximately hourly intervals through the blood collection system described above. Blood samples (approximately 1-3mL) will be transferred to small (3 mL) vacutainer tubes and centrifuged; the resulting plasma will be pipetted into vials and frozen until analysis. Melatonin assays will be performed by Solidphase (Portland, ME) using a commercially-available radioimmunoassay kit.

Intravenous Glucose Tolerance Test (IVGTT; insulin-modified). An insulin-modified IVGTT will be carried out on Days 7 and 15 after an overnight fast [13] and assessment of REE. Blood samples will be drawn via an intravenous catheter every 5 min for 20 min starting at T=-20 min. At time 0 (time of usual breakfast), 0.3 g/kg glucose will be administered over 1 minute as an intravenous bolus via an intravenous catheter in the opposite (non-sampling) arm. Blood samples will be taken at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 21, 22, 24, 26, 28, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, and 180 min. At time +20 min, 0.02U/kg human insulin will be administered intravenously over 1 minute. Minimal Model Analysis [102] will be used to assess insulin sensitivity (S_I) and glucose effectiveness. Acute insulin response (AIRg) will be calculated as the average incremental plasma insulin concentration over the first 20 minutes after the glucose bolus (and before the insulin administration) [22].

Continuous glucose monitoring. As supplementary/exploratory data, we may use an Abbott FreeStyle continuous glucose monitor (Abbott Laboratories) to document glucose levels every 15 minutes throughout the inpatient study. If so, a physician or nurse will insert an interstitial glucose sensor in the participant’s arm on Day 4, and it will be replaced approximately every 10 days or as needed throughout the end of the study. The sensor will thus be worn throughout the study to collect continuous glucose data (one measurement every 15min). The data will be downloaded at the end of the collection period using a reader device. This information will provide a dynamic view of changes in glucose levels throughout the study, for example in near-darkness while awake.

Biomarkers of sleep loss (panel). This panel will be collected for exploratory analyses, and reflects known and emerging blood and/or saliva constituents that are candidates quantifying the health impact of sleep loss on systems including glucose regulation (HbA1c, fasting insulin and glucose [HOMA index], free salivary cortisol levels), thyrotropic axis function, lipid metabolism (NEFA release during IVGTT), and peripheral markers of energy balance (leptin and ghrelin). Samples may be collected before the start of each IVGTT and assayed for: Lipid Panel, HbA1c, insulin-like Growth Factor 1 (IFG-1), thyroid stimulating hormone (TSH), free thyroxine (T4), tri-iodothyronine (T3), electrolytes, adiponectin, aldosterone, plasma renin activity (PRA), and/or C-reactive protein (CRP).

Plasma lipidomics. Profiling analysis involves giving primary consideration to a collective network of metabolites [levels of long-chain highly un-saturated lipids and short-chain, highly saturated lipids], and focusing on the determination of categorical differences between physiological states [baseline vs. after sleep restriction, with and without ALAN]. Our liquid chromatography-mass spectrometry (LC-MS) method enables separation of lipid classes as well as both qualitative and quantitative detection of >600 structurally identified individual lipids in biological samples.[36-47] Data are acquired using high resolution full scan MS and high energy collisional dissociation MS/MS. The method was evaluated for efficient separation and detection in both positive and negative ionization mode using standards spanning 6 lipid classes. The approach is currently able to detect fatty acids (FA), sterols (ST), lysophosphocholines (Lyso-PC), phosphotydilcholines (PC), phosphotidylethanolamines (PE), lysophosphoethanolamines (Lyso-PE, phosphotydylglycerols (PG), sphinglipids (SL), diacylglycerols (DG), phosphotidylserines (PS), quinones such as ubiquinone, acyl carnitines, cholesterol esters, cholesterol sulfate, sulfatides, hexCers and and other lipid subclasses. We will begin by generating lipidomic profiles of all samples, assess levels of individual lipids in baseline vs sleep restricted conditions (separately for each light/no light intervention at this phase) looking at both absolute and relative signal (ie., mass effect of lipids and relative occupation of total lipid pool). For the triglycerides, we will additionally assess the set Rhee et al.[1] associated with diabetes risk [e.g., increased risk with TG 44:1; TG 46:1; TG 48:0; TG 48:1; TG 50:1; TG 52:1; decreased risk with TG 56:9, TG 58:10, TG 60:12]; we will then look at total carbons and total double bonds in each species in the triglyceride and phospholipid pools vs. the sleep restriction phenotypes. We will assess the within-person stability of each lipid within the baseline data itself. Finally, we will test the general hypothesis.

Total fat mass and BMI. Body Mass Index (BMI) will be assessed using measured height and weight, and waist and hip circumference. A DXA scan (Discovery W Dual-energy X-ray Absorptiometry Scanner, Hologic, Bedford MA) will be carried out at the BWH Bone Density and Body Composition Research Core to measure total fat mass prior to the start of the study and again at the end of the study. We will look for changes in fat mass, especially visceral/trunk adiposity, and will further be able to determine whether there are changes in muscle mass with the low levels of activity in controlled laboratory conditions.

Saliva sample collection. Saliva will be collected approximately every waking hour as a backup to the blood samples collected for melatonin [100]. Participants are instructed to not eat anything within 30 minutes of a sample, and may be asked to rinse their mouth with water after eating. The exact time of collection will be documented and the sample will be frozen at -20ºC for later assay (if needed).

Oral microbiome sample collection. Studies have found that oral microbiota are impacted by delayed meal timing, which is common in shift workers and people obtaining insufficient sleep. Additional saliva samples and/or tongue swabs may be collected approximately once per day throughout the study for salivary/oral microbiome analysis. The exact time of collection will be documented and the sample will be frozen at -80ºC for later assay.

Genetic sample collection and whole genome sequencing. Given the findings that genetic polymorphisms are associated with sleep, circadian, and metabolic physiology, we will collect genetic material from each participant for genome-wide analysis as well as future research. A total of 30mL of blood will be collected from each participant either at the time of discharge from the study or during a screening visit. Blood samples will be sent to the Mass General Brigham Biobank where DNA will be extracted, purified, and stored [49, 118, 119]. A portion of each sample may be sent to the Genomics Platform at the Broad Institute where standard whole genome sequencing (WGS) will be performed.

Objective alertness assessment. At approximately hourly intervals throughout the scheduled wake episodes, participants will take a modified Karolinska Drowsiness Test (KDT [107, 120, 121]) to evaluate objective alertness. The KDT is a standardized procedure for providing a highly sensitive, objective EOG (incidence of slow eye movements and eye blink rates) and EEG measures to assess alertness. During the KDT, participants are asked to remain still while fixing their gaze for ~4 minutes, avoiding frequent eye blinks, eye movements and body movements. This test allows for reliable collection of EEGs of high signal quality. These objective measures of sleepiness have been shown to vary with circadian phase and duration of wakefulness[122-126]. Our staff and the technical staff of the CCI routinely conduct such recordings. EEG will also be recorded throughout the wake episodes to document wakefulness [107].

Subjective alertness assessment. Throughout the study, participants will complete computer-administered alertness assessments at approximately hourly intervals when they are awake. These will include the Karolinska Sleepiness Scale (KSS) and a Visual Analog Scale (VAS). The KSS requires the participant to select a number on a scale from 1 to 9 spanning the range from very sleepy to very alert. The task takes less than 1 minute, and KSS scores have been shown to oscillate with the core body temperature and plasma melatonin rhythms [109-111]. Visual Analogue Scales (VAS) consist of a horizontal line drawn on the computer display with each end of the line labeled with the extremes of a subjective continuum, e.g. sleepy-alert. Participants indicate a position on the line that best describes how they feel at that moment. The task takes less than 1 minute to complete, and subjective estimates have been shown to vary with circadian phase and sleep loss [112-115].

Neurobehavioral performance assessment. Throughout the study, participants may be asked to complete additional computer-administrated performance test batteries several times per wake period. This battery may include the following tests:

- **Psychomotor Vigilance Task** (PVT) to assess **sustained attention performance**. The PVT is a test of visual reaction time (RT) in which the subject is asked to maintain the fastest possible RTs to a simple visual stimulus for ~10 minutes. The inter-stimulus interval varies randomly between 2-10 seconds.
- **A Calculation Performance task** (a measure of **cognitive throughput**), modeled after one described by Klein et al.. This task presents the subject with a series of randomly generated pairs of 2-digit numbers and the subject's task is to sum as many pairs as possible in the allotted four-minute time interval.
- **Visual search tasks** to investigate **attentional selection**, including a **conjunction search** task in which the subject must look for a target (red vertical bar) among a variable number of distractors (green vertical and red horizontal bars), a **serial search** task in which the subject must look for a target “5” among a variable number of distractors (“2”s), and/or the **Comparative Visual Search task** (CVS) which includes a component of working memory. The task requires subjects to compare two side-by-side images (a target and a copy) consisting of an array of triangles pointing left and right. In the “copy” version, the two images are exact copies with the exception that one of the triangles in the target diagram is pointing in the wrong direction; in the “mirror” version of this task, the two images are mirror images of one another, and again the subject’s task is to locate the triangle that is pointing in the wrong direction. Thus, the subject must remember which parts of the copy array he is comparing with the target and the direction of the triangles within it, and remember which part of the target array he has already searched (working memory), as well as searching for the target triangle.
- **The Stroop color-naming task**, a test of executive function. This test involves the inhibition of a prepotent response, and performance on the Stroop has been demonstrated to slow with sleep loss. Our version of the Stroop includes three types of stimuli: matching word and color (congruent), non-word and color (neutral), and mis-matched word and color (incongruent)[69].
- **A Probe Recall Memory** (PRM) test, a test of short-term memory for unassociated pairs of words, consisting of a 30 second learning phase and then recall (either immediately or after a fixed delay). During the learning phase, the subject is required to memorize ~6 pairs of unassociated words. During the delay phase, the subject is administered an unrelated cognitive task. In the recall phase, the subject is required to recall the second word of each pair in response to the computer’s presentation of the first word or to answer yes or no in response to pairs of words presented on the screen.
- **A Face-Name Association task** (F-N) in which the subject is presented with a series of photographs of

individuals they do not know during the learning phase, and each photograph has a name associated with it. They are tasked with remembering the name of each face. The recall phase of this test occurs 1 or more hours later. Performance on this task activates the hippocampus and left inferior prefrontal cortex., and is impaired in dementia and MCI.

- Matrix Reasoning Test (MRT), in which the subject is shown a series of twelve 3 x 3 matrices in which 8 of the 9 squares of the matrix are populated with an image and the 9th square (in the lower right of the matrix) is left blank. Subjects are also presented with a 2 x 4 matrix that contains the correct image in addition to 7 incorrect images and must select the image that completes the 3 x 3 matrix.
- Balloon analogue risk task (BART), which assess risk-taking behavior. Participants are presented with 30 trials in which they must choose to either inflate an animated balloon presented on the left side of the screen or collect a reward. The reward can be collected at the beginning of the trial (without inflating the balloon) or after any number of pumps which inflate the balloon by increments. The reward that can be collected on each trial is proportional to the final size of the balloon; however, if the balloon is overinflated and pops, the reward for that trial is lost.
- Tests of emotional memory and/or perception, such as the Emotional Memory Task in which subjects view images, slides, or scenes ranging in emotionality from standardized and validated stimuli sets (e.g. International Affective Picture System; IAPS or Nencki Affective Picture System; NAPS) and are asked to rate and/or remember the scenes for later testing; the Emotional Trade-off Task, in which subjects view images consisting of an object, either emotional (i.e. a car wreck) or neutral (i.e. a taxi), composited onto a background (i.e., a city street) and are later shown objects and backgrounds in isolation and asked to remember whether or not they saw an identical or similar item; and the Morphed Faces Task, where subjects view a series of pictures created by separately morphing a neutral face with 3 different emotional faces (Angry, Happy and Sad) and are asked to identify each face's emotional category, as well as rate their interpretation of its intensity and valence (negative to positive).

Voice recording collection. During scheduled wake episodes a speech task may be administered in addition to each scheduled neurobehavioral test battery proposed above. The speech task will be administered via computer and will take less than 2 minutes in total. Each speech task begins with a prompt to the participant that their voice will be recorded throughout the task, and to make sure they are sitting facing the computer monitor and microphone. They are then asked to read a list of 10 sentences from the Harvard Sentences corpus [IEEE Recommended Practice for Speech Quality Measurements. IEEE Trans Audio Electroacoust. 1969;17(3):225-246. doi:10.1109/TAU.1969.1162058].

Steps for a single speech task:

1. The computer program first generates a random 3-digit number and picks a list (10 sentences) from the Harvard Sentences for the current assessment.
2. One sentence at a time is presented to the subject on the computer monitor. After reading out the sentence, the subject presses the spacebar on the keyboard to proceed to the next sentence.
3. After recording the 10 sentences by repeating Step 2, the 10 recordings are saved separately as waveform audio files (WAV) under a folder named by the random 3-digit number from Step 1.

The speech of the subjects will be recorded by a Shure VP64A omnidirectional microphone or equivalent (frequency response: 50 to 12,000 Hz) mounted on a desktop microphone stand. The microphone is connected to the computer via a USB to XLR adapter and cord extensions. The height and horizontal extension of the stand are adjusted to hold the microphone close to the lips of the subject when sitting at the desk in their study room in the lab. The microphone is covered by a standard windscreen.

While previous studies used different text corpora and languages when recording human voice, most have emphasized phonemically balanced texts [Bagnall AD, Dorrian J, Fletcher A. Some Vocal Consequences of Sleep Deprivation and the Possibility of "Fatigue Proofing" the Voice With Voicecraft® Voice Training. Journal of Voice. 2011;25(4):447-461. doi:10.1016/j.jvoice.2010.10.020; Icht M, Zukerman G, Hershkovich S, et al. The "Morning Voice": The Effect of 24 Hours of Sleep Deprivation on Vocal Parameters of Young Adults. Journal of Voice. 2020;34(3):489.e1-489.e9. doi:10.1016/j.jvoice.2018.11.010; Amato F, Rechichi I, Borzi L, Olmo G. Sleep Quality through Vocal Analysis: a Telemedicine Application. In: 2022 IEEE International Conference on

Pervasive Computing and Communications Workshops and Other Affiliated Events (PerCom Workshops). IEEE; 2022:706-711. doi:10.1109/PerComWorkshops53856.2022.9767372] and vowel pronunciations [Barron DS, Heisig S, Agurto C, et al. Feasibility Analysis of Phenotype Quantification from Unstructured Clinical Interactions. Computational Psychiatry. 2022;6(1):1. doi:10.5334/cpsy.78; Rahman W, Lee S, Islam MS, et al. Detecting Parkinson Disease Using a Web-Based Speech Task: Observational Study. J Med Internet Res. 2021;23(10):e26305. doi:10.2196/26305]. Thus, our speaking task involves reading one list from the Harvard sentences [IEEE Recommended Practice for Speech Quality Measurements. IEEE Trans Audio Electroacoust. 1969;17(3):225-246. doi:10.1109/TAU.1969.1162058] each time. There are 72 lists of Harvard sentences, each containing 10 sentences. The computer program will pick a distinct list from the corpus every time the speech task is performed so subjects do not become familiar with the text and degrade their pronunciation by reading too fast or rumbling.

Stool sample collection. The gut microbiome is thought to be a potential candidate mechanism underlying the effects of sleep and circadian disruption on metabolic health. Therefore, we may collect stool samples at several points throughout the study for use in microbiome analysis. On specific study days, participants may be instructed to notify the staff at their next bowel movement so that they may be provided with a collection kit for stool sampling. The exact time of sampling will be documented and the sample will be frozen at -20ºC for later assay.

Breath sample analysis. We may use selected ion flow tube mass spectrometry (SIFT-MS) to collect samples of breath for metabolomic analysis. The Syft T007 Voice200 Ultra performs Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) and is a technology that has the ability to identify breath biomarkers accurately and reliably. Syft is sensitive (up to parts per billion), specific (~1000 known compounds), fast (sampling time <1 minute), and inexpensive (requiring only plastic straws and water to acquire a sample). At selected intervals during the study, the participant may be asked to take part in a breath collection segment. They may be asked to brush their teeth with water and/or rinse their mouth, and then be seated and remain seated for the 20-30 minute collection. During the sample collection segment, the participant will be asked to inhale through their nose and exhale into a straw connected to the Syft T007 Voice200 Ultra for ~2.5-minute sampling segments.

Private Identifiable Information. All data for this study will be collected and recorded using a study code, rather than individually-identifying information. This is a routine practice in our laboratory and at the CCI, and we therefore have systems in place to assign a study code upon enrollment into the protocol and to use study codes on automated data collection systems in the CCI (for the activity data, PSG data, alertness and performance data, biospecimen, breath samples).

Some identifiable information about the study will be collected. Tests and questionnaires from the screening process do include identifiable information. The Division of Sleep and Circadian Disorders has procedures in place to maintain the confidentiality of that information in areas with limited access. During the inpatient study at the CCI the participant is considered a patient at the hospital, so record of their participation and limited records of their visit (admission and discharge notes, CCI RN and attending MD notes, daily vital signs, clinical blood test results) are including in their electronic medical record. No study data are kept in the medical record. As outlined in the Human Subjects section below, all staff members of the Division of Sleep and Circadian Disorders and the CCI are trained in HIPAA compliance, research ethics, and privacy issues, and are routinely re-certified.

VI. Biostatistical analysis

Intent-to-treat analysis and per protocol analysis will both be performed. The amount and patterns of missing data, if any, will be characterized. We will use all available data points in the mixed-effects model. The study will be monitored routinely for issues of data quality, study conduct, and adverse events. We do not plan to do interim analysis. Mediation analysis will be performed to study the relationship between extended-duration ALAN exposure, duration of melatonin secretion, and the primary outcomes (insulin sensitivity and glucose tolerance). Paired t-test (or Wilcoxon signed-rank test) will be used to compare the change from baseline in insulin sensitivity (SI), acute insulin response (AIRg), glucose effectiveness, glucose AUC, insulin AUC, and the duration of the melatonin profile after exposure to sleep restriction with and without ALAN. Linear mixed models will also be used to compare the change in these outcomes from baseline to after exposure to sleep restriction with and

without ALAN in case of missing data and with potential covariates, such as age and sex.

Insulin sensitivity (S_I) (Specific Aim 1). An insulin-modified IVGTT will be carried out on Days 4 and 11 after an overnight fast [13]. Blood samples will include pre-test baseline samples. At time 0 (time of usual breakfast), 0.3 g/kg glucose will be administered over 1 minute. Blood samples will be taken every minute for the initial 6 minutes, every ~2 minutes for the following ~20 minutes. At time +20 min, 0.02U/kg human insulin will be administered intravenously over 1 minute. Blood samples will continue to be taken every 5 minutes for the following half hour, every 10 minutes for the next ~half hour, and every 20 minutes for the next 1.5 hours. Minimal Model Analysis 3 will be used to assess insulin sensitivity (SI).

Acute insulin response (AIRg) (Specific Aim 1). An insulin-modified IVGTT will be carried out on Days 4 and 11 after an overnight fast [13]. Blood samples will include pre-test baseline samples. At time 0 (time of usual breakfast), 0.3 g/kg glucose will be administered over 1 minute. Blood samples will be taken every minute for the initial 6 minutes, every ~2 minutes for the following ~20 minutes. At time +20 min, 0.02U/kg human insulin will be administered intravenously over 1 minute. Acute insulin response (AIRg) will be calculated as the average incremental plasma insulin concentration over the first 20 minutes after the glucose bolus (and before the insulin administration) [22].

Glucose effectiveness (Specific Aim 1). An insulin-modified IVGTT will be carried out on Days 4 and 11 after an overnight fast [13]. Blood samples will include pre-test baseline samples. At time 0 (time of usual breakfast), 0.3 g/kg glucose will be administered over 1 minute. Blood samples will be taken every minute for the initial 6 minutes, every ~2 minutes for the following ~20 minutes. At time +20 min, 0.02U/kg human insulin will be administered intravenously over 1 minute. Additional blood samples will be drawn at minutes 21, 22, 24, 26, 28, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, and 180. Minimal Model Analysis [102] will be used to assess glucose effectiveness.

Glucose AUC in response to a standardized Mixed Meal (Specific Aim 2). Post-prandial glucose response will be assessed at breakfast following an overnight fast [32] at baseline (Day 3) and at the end of the sleep intervention segment (Day 10). The meal will be identical at each test and the participant will be required to eat all of the meal within 30 minutes. Blood samples will include pre-meal baseline samples as well as post-prandial samples at 10-min intervals for the first 90 minutes following the meal, then at 30-min intervals for the next 90 minutes. The glucose area under the curve for time 0 to 120 minutes will be calculated using the trapezoidal method.

Insulin AUC in response to a standardized Mixed Meal (Specific Aim 2). Post-prandial insulin response will be assessed at breakfast following an overnight fast [32] at baseline (Day 3) and at the end of the sleep intervention segment (Day 10). The meal will be identical at each test and the participant will be required to eat all of the meal within 30 minutes. Blood samples will include pre-meal baseline samples as well as post-prandial samples at 10-min intervals for the first 90 minutes following the meal, then at 30-min intervals for the next 90 minutes. The early phase insulin area under the curve for time 0 to 30 minutes will be calculated using the trapezoidal method. The late phase insulin area under the curve for time 30-120 minutes will also be calculated using the trapezoidal method.

Analysis of circadian phase (Specific Aim 3). Plasma melatonin will be collected throughout the circadian phase assessment procedures on Days 3-4 and 10-11 for assessment of circadian phase and amplitude. Two measures of melatonin phase can be derived from the data collected in the proposed studies. In the first method, the dim light melatonin onset (DLMO), the time at which the plasma levels reach 10 pg/ml, will be determined [130]. To account for the wide variation between individuals, a second method of estimating melatonin phase, a relative dim light melatonin onset ($DLMO_{25\%}$) will also be estimated, computed as the time at which the plasma melatonin rhythm crosses a threshold of 25% of its fitted value [98, 100] derived from the entire baseline time series. Linear interpolation between adjacent samples will be used to determine DLMO and $DLMO_{25\%}$. The same threshold derived from the melatonin data on the baseline profile will be used to re-assess $DLMO_{25\%}$ on the re-assessment (at the end of the sleep intervention). In addition, the area under the

curve (AUC) of the plasma melatonin profile from the baseline and re-assessment profiles will be calculated using the trapezoidal method.

Analysis of voice recording (Exploratory Aim 8). Human vocal parameters will be collected during specified wake times. Power spectral density, intensity, pauses, reading mistakes, and automatic speech recognition (ASR) system errors are extracted from each audio file containing one sentence recording. These features will be aggregated at various scales (over each list or subject). Different acoustic analysis techniques and computer programs will be used to extract these features in a retrospective manner. The table below lists our proposed features.

Feature	Note
Duration (seconds) and percentage of voiced segments	
Duration (seconds) and percentage of vocalic segments	
Number, duration (seconds), and location of pauses	Natural and unnatural pauses
Reading mistakes (counts)	Stumbling, paralexia, deletion, and addition
ASR errors (counts)	
Power spectral density (square of amplitude)	Mean, variance, min, max, and slope
Intensity analysis (decibel)	Mean, variance, min, max, and slope
Jitter (seconds), shimmer (seconds), and HNR (%)	Provided by the Praat Software

After preprocessing and extraction, vocal features will be decorrelated with confounding factors, including age, sex, BMI, and other demographic information of subjects. Any feature correlated to confounding factors will be replaced by the residue of a multivariate linear regression approximating the value of the feature from confounding factors. One-way ANOVA will be performed to examine if vocal features are significantly different between recordings from different sleep-wake conditions (baseline, restricted sleep with extended duration ALAN, and restricted sleep without extended duration ALAN). Mann-Whitney U tests will be performed to compare between conditions. For repeated vocal parameter measurements during the same experimental segment, they will be aggregated and averaged over the segment duration to achieve global-level comparison.

Sample size and power calculation

We will consider three primary outcomes: insulin sensitivity for Specific Aim 1; glucose AUC for Specific Aim 2; and duration of melatonin secretion for Specific Aim 3. Sample size and power calculations are based on results from our previous studies. In the study of one week of sleep restriction to 5 h/night[13], we observed a ~28% decrease in insulin sensitivity as assessed by ivGTT. We anticipate a similar effect on insulin sensitivity in participants assigned to the Sleep Restriction with extended duration Artificial Light At Night (ALAN) group in the proposed study, given the identical conditions of 1 week of sleep restriction. In a separate study, no impairment of glucose tolerance was observed in a group of participants exposed to sleep restriction without extended duration exposure to ALAN (See Preliminary Studies, Fig. 3). With 20 subjects, we have 83% power (α=0.05/3=0.0167, 2-sided paired t-test) to detect as little as a 13% difference between the conditions in the change from baseline (18% change with ALAN vs. 5% change without ALAN) in insulin sensitivity (assuming the common standard deviation (SD) of the change is 16%). This should be more than adequate to detect the difference in insulin sensitivity we expect to observe in the proposed study. For Specific Aim 2, with 20 subjects, we estimate that we have 83% power (α=0.0167, 2-sided paired t-test) to detect as little as a 3.65% difference between the conditions in the change of glucose AUC (assuming common SD= 4.5%). This should be more than adequate to detect the difference in glucose AUC we expect to observe, given that we have previously observed an over 20% change in glucose AUC following a prior history of recurrent circadian disruption combined with chronic sleep restriction[32].

For Specific Aim 3, we estimate that we will have 84% power (α=0.0167, 2-sided paired t-test) with 20 subjects to detect a difference as small as 40 minutes (0.66 hrs) in the duration of melatonin secretion (1 hr with ALAN vs. 20 minutes without ALAN, assuming common SD=48 minutes). This should be more than adequate to detect the expected difference in melatonin secretion, given that we previously showed that evening exposure to room light shortened the duration of melatonin secretion by about 90 minutes (compared to dim lighting), even when participants remained in darkness for 8hrs/night during sleep[94]. Because participants in the current proposed

study will be exposed to room light for an extra 5 hrs/day in the sleep restriction with ALAN condition, we expect to see a more than 3-hour difference in the duration of melatonin secretion with and without ALAN.

For Exploratory Aim 4, we will consider two primary outcomes. We estimate that we have 85% power (α=0.025, 2-sided paired-sample t-test) to detect an 20% difference in the change (25% with ALAN, SD=28% vs. 5% without ALAN,SD=6%) in levels of long-chain, highly unsaturated triglycerides, which have been associated with insulin sensitivity. Moreover, we estimate we will have 85% power to detect a difference as little as 11% in the change (5% with ALAN, SD=6% vs. 18% without ALAN,SD=19%) in levels of short-chain, highly saturated triglycerides, levels of which have been associated with increased diabetes risk.

VII. Risks and Discomforts

Potential Risks to Subjects Associated with Each Study Intervention, Procedure or Interaction.

Risks of blood sampling procedures.

Common Risks

There may be some discomfort or bruising on initial insertion of the IV catheter into a vein but wearing the catheter should not be painful.

Occasionally, there is a black and blue mark at the site of the IV insertion, which may last a couple of weeks; and, rarely, a small scar may remain permanently at the venipuncture site.

Occasionally, mild discomfort may occur from the tube in the vein. If this happens, we will either reposition it or remove it entirely, asking the participant's permission before any subsequent reinsertion.

There may be a minor skin rash or reaction (contact dermatitis) to the sterile tape used to hold the catheter in position. Hypoallergenic tape will be used as necessary.

The amount of blood drawn should not significantly alter blood volume, although there may be a small decrease in the hemoglobin level. The total volume of blood drawn over the course of the month-long study will be no more than 2 pints (946 mL).

Uncommon Risks

There is the possibility that the participant may faint during or after the IV insertion procedure.

There is a rare possibility of developing a small blood clot, inflammation, or local infection around the vein where the catheter is inserted. In rare cases, a generalized infection can spread through the bloodstream as a result of the IV catheter.

There may be some side effects from the use of heparin, such as bleeding or allergy. The amount of heparin used is much less than what would be used therapeutically to prevent clotting.

Risks of PSG collection.

Common Risks

There may be a minor skin rash or reaction (contact dermatitis) at the sites where the electrodes are placed in response to the conducting gel, the collodion used to adhere the electrodes to the hair, or to the sterile tape used to hold the electrodes to skin on the face.

Risks of insulin-modified IVGTT.

Common Risks

There is a second IV inserted for the IVGTT, and the same risks apply to it as to the sampling IV described above.

During the IVGTT, the sampling IV line is placed in a hand/wrist vein and this hand may be placed in a heated box to allow for easier sampling (140 degrees Fahrenheit). This may cause sweating and slight swelling of the hand, and before the IVGTT procedure begins the subject will need to take off any rings that he/she normally wears on fingers of the hand being used. The temperature of the heated box and the subject's hand will be closely monitored throughout the entire IVGTT procedure. The subject is free to remove the hand from this box if there is discomfort during the procedure.

Uncommon Risks

Intravenous glucose infusion will raise blood glucose levels mildly. There is a rare risk of tissue extravasation if glucose is pushed too quickly through a peripheral IV line. Accordingly, the current IVGTT protocol used on the CCI requires a slow IV push method by an experienced provider who will monitor for pain/tenderness at the infusion site. A specially trained clinician will perform the IVGTT, which have been performed over 100 times in healthy individuals by our research core.

Insulin is injected intravenously in a quantity related to body weight. Insulin lowers blood glucose and can cause a lowering of circulating potassium levels. In lowering glucose levels, there is risk of hypoglycemia. Symptoms of hypoglycemia are reversed rapidly with correction by either oral or IV glucose administration. The IVGTT will be carried out by a specially-trained clinician, and a second nurse will be present throughout the procedure. A symptom check is performed at 30, 60, 120, and 180 minutes after glucose infusion. Blood glucose safety checks are performed 20 minutes before the start of the procedure, as well as 160 min and 180 min after glucose infusion. An “Emergency Medication Kit” is ordered at bedside for each IVGTT that consists of: glucose tablets, IV glucose, IM glucagon, IM epinephrine, IV diphenhydramine, IV hydrocortisone, and acetaminophem. A CCI Standard Operating Procedure dictates threshold values for glucose and corresponding treatment options.

The dose of insulin used in this procedure is small and would not be expected to lead to low potassium levels. As a precaution, the participant’s potassium level will be checked to ensure it is within normal range.

Radiation risks

Body composition measurement involves radiation exposure from a scan of the whole body using dual x-ray absorptiometry or DXA. The radiation exposure from these scans is less than 1% of the normal, annual background radiation exposure. The risk from radiation exposure of this magnitude is too small to be detected in this study's population.

Risks of receiving study visit reminders by text message

Text messages are not encrypted, and thus there is a risk of a loss of privacy. To minimize this risk, we will adhere to the principles of minimum necessary; thus, study-initiated text messages will not contain participant names or other identifiers, sensitive information that could expose financial risk to individuals (social security numbers, driver license numbers, credit card numbers, etc), results of any clinical tests conducted for the purpose of research, or potentially sensitive information about the study visit. Additionally, participants who consent to receiving text messages will first be sent a welcome message by text that requests the participant to respond “yes” to indicate their preference to continue to receive research texts.

Other Study Risks.

Common Risks

Staff in the CCI metabolic kitchen will calculate a menu cycle based on the individual participant's preferences that meets the diet order and is designed to maintain each participant’s weight and adhere to the planned macronutrient content. The participant will be required to eat all the foods they are served at each meal. Furthermore, on each of the occasions that a Mixed Meal Response test is given, the content of that breakfast, and the food on all the meals on the prior day, will be exactly the same. The participant may find this to be unappealing, but they will be consuming foods from a menu of study-compatible options that they choose.

For all scheduled sleep episodes, and for the 2.5 hours before and after each restricted sleep episode in the Sleep Restriction without ALAN condition, the participant will be restricted to bed and will not be allowed to get out of bed. During scheduled sleep episodes, the room will be completely dark. The participant will not be allowed to leave the bed or turn on the lights during the scheduled sleep episodes, even if they find it difficult to remain asleep. They will have to use a bedpan or urinal as needed during this time.

For the assessment of Resting Energy Expenditure and the Mixed Meal Response baseline tests, the participant will be restricted to sitting quietly in bed from the time that they are awakened until the blood sampling from the Mixed Meal test is complete approximately 4 hours later. For the same tests carried out at the end of each sleep restriction intervention condition, participants will awaken 2.5 hours before usual, and thus will remain sitting in bed for approximately 6.5 hours. The participant will have to use a bedpan or urinal as needed during this time.

For the 2.5 hours before and after each restricted sleep episode in the Sleep Restriction without Extended Duration ALAN condition, the lighting in the room will be very dim. This will make it difficult for the participant to

read or do other visual tasks. There will be a trained staff member in the room with them to converse, play a board game, etc.

There will be times when the participants will feel extremely sleepy. There are no known permanent adverse effects from chronic sleep restriction for a limited time such as the 2 weeks in the proposed studies. The participant may experience headache, nausea, upset stomach, or feel very irritable or frustrated when they are very sleepy and not allowed to go to sleep**.** The participant will be informed that they may withdraw from the study at any point that they feel this sleepiness is too extreme, but they will be required to sleep for at least 4 hours before being discharged from the study.

The assessment of resting metabolic rate will take approximately 20-30 minutes, during which time the participant will be asked to sit underneath a ventilated hood. This may be unpleasant for some individuals. If the subject/patient feels that the discomfort is excessive he/she can choose to terminate the study.

Body composition measurement will be done by a scan of the whole body using dual x-ray absorptiometry or DXA. This may involve the minor discomfort of lying still for 5 minutes.

Mild discomfort may occur when the continuous glucose monitor sensor is first placed. It should not be uncomfortable while wearing it. Mild skin irritation may occur when the sensor is removed at the end of the sampling segment.

Mild irritations such as edema, rash, bleeding, itching and infection may occur from the site where the continuous glucose monitor sensor is placed. In our study, the sensor will be placed by a nurse after cleaning the skin in order to minimize the risk of infection. The site will be checked daily by a CCI nurse and the placement moved if edema, rash, itching, or bleeding occurs.

Participants may feel uncomfortable answering some of the questions that are part of the pre-study screening process.

Participants may feel uncomfortable answering some of the questions that are part of the hospital admission process.

Participants may feel uncomfortable viewing the emotional imagery used in some of the performance tests.

Participants may feel uncomfortable having a staff member in their room throughout the entire wake episodes during the week of sleep intervention. They will be informed of this monitoring ahead of time, and can choose to end the study early if they feel they cannot tolerate it.

Because of the sleep loss experienced during the study, participants will be advised that they will likely be sleepier than usual after the study is complete, and that this may last for several days. They will be advised to allow themselves extra time for sleep and to avoid situations that put them at risk for an accident, such as driving an automobile or operating heavy machinery.

In order to minimize the risks associated with jet-lag type symptoms, participants will be counseled prior to discharge on how to readjust to their regular sleep-wake cycle if they should experience jet lag symptoms. In our experience, most potential participants have either experienced jet-lag in the past, and/or have kept an irregular schedule in the past, and thus can anticipate what these symptoms will be like.

VIII. Potential Benefits. Although there will be no direct physical benefit to the participant resulting from participation in this study, we will make known to the participant, upon conclusion of the protocol and analysis of the data, some of the information we have gathered from the physiological testing. This information may help the participant develop a more compatible daily schedule for sleeping, which in turn may result in improved well-being. We will also provide the participant with copies of the screening blood and urine tests, ECG, and physical examination form if they request them.

There is a chance that the pre-study screening will reveal some medical abnormality. This information will be conveyed to the participant at once, together with a recommendation of a local clinic or physician from whom to seek treatment.

The research has the potential to benefit society more widely. This study is designed to evaluate the extent to which circadian disruption induced by longer exposure to artificial light at night contributes to the impairment of glucose metabolism associated with sleep restriction. By the end of the 20th century, Americans were sleeping on average 2 hours less per night than they were at the beginning of the 20th century. In fact, recent polls conducted by the National Sleep Foundation indicate that young and older adults alike report sleeping less than what is recommended [127, 128]. More than a third of adults report sleeping less than 7 hours per night, with

approximately 15% of adults reporting less than 6 hours of sleep per night on a regular basis. While sleep researchers have begun to understand the consequences of acute sleep loss on alertness and performance, far less is understood about the consequences of chronic insufficient sleep [129] or how to mitigate those impacts. While the adverse metabolic consequences of chronic sleep insufficiency are now widely appreciated, the role played by light exposure at night on those metabolic changes is not understood. **By understanding the contribution of extended light exposure on the adverse metabolic changes associated with chronic sleep restriction, we can design and test strategies to minimize or eliminate those risks.**

Experimental studies have demonstrated that chronic sleep restriction impairs glucose metabolism, consistent with epidemiologic studies that show strong links between short sleep, metabolic syndrome, and diabetes. However, less is known about whether one of the mechanism(s) by which insufficient sleep impairs glucose metabolism is light exposure at night. Given that sleep restriction is typically accompanied by longer exposure to artificial light at night, which is known to disrupt circadian rhythmicity, we plan to evaluate the extent to which circadian disruption induced by exposure to extended duration artificial light at night contributes to the impairment of glucose metabolism associated with sleep restriction. **This knowledge can be used to design and test countermeasures and mitigation strategies to minimize the adverse metabolic consequences of chronic sleep restriction.**

IX. Monitoring and quality assurance

Overall Framework for Safety Monitoring. This study will be conducted under the regulations and oversight of the Mass General Brigham (MGB, formerly Partners HealthCare System) Human Subjects Committee, required for all studies carried out at the Brigham and Women's Hospital. The PI (Dr. Czeisler) will be responsible for ensuring all Human Subjects regulations and policies are followed, and for reporting to the Human Subjects Committee, as required. Furthermore, the study must be approved by the Harvard Catalyst Clinical and Translational Science Center Scientific Advisory Committee before it can be conducted on the BWH CCI. All study personnel involved in the participant recruitment, study execution, data collection, and data analysis at BWH have completed Human Subjects training as well as training in the procedures required by HIPAA, and must annually certify that they will comply with all such regulations. Drs. Czeisler, Duffy, Kristal, Saxena, Scheer, Wang, Williams, and Yuan have completed additional training in Medical Ethics as required by their appointments at the Harvard Medical School.

Individual(s) or Group Responsible for Monitoring. The PI will be responsible for carrying out the DSMP. Data and safety will be monitored by the PI, with assistance from the study personnel, attending physicians, and CCI nurses, and reported to the IRB as required. The procedures proposed in this study have been in use in the PI's research program for the past 40 years, and his team as well as the CCI research nurses and technicians have used the same procedures in hundreds of studies over the two decades since the CCI-IPM opened.

Information to be Monitored. The following aspects of the study will be monitored: enrolment, including accrual and retention and tracking by sex and race/ethnicity; protocol non-compliance; data completeness; and adverse events.

Specific issues to be monitored include: adherence to the sleep schedule, meal diary, and wearing of the actigraphy monitor in the pre-study phase; collection of the blood samples and compliance with the conditions for circadian analysis; conduct of the Mixed Meal response procedure including collection of the frequent blood samples; conduct of the intravenous glucose tolerance tests including collection of the frequent blood samples, and occurrences of hypoglycemia; collection of the PSG data each night of the inpatient study; collection of EEG and EOG data during the wake episodes to verify wakefulness throughout all scheduled times; collection of vigilance and subjective sleepiness data during the scheduled wake times; collection of voice recordings during scheduled wake times, including suitable volume and clarity for proposed audio analyses, enough sentence completeness, and the correct file format (WAV) and storage location; common and uncommon risks as described elsewhere.

Procedures for Monitoring and Frequency of Review. During the inpatient study conducted on the CCI, participants will be supervised by CCI nursing staff and technical laboratory personnel to monitor participant

safety. The CCI nursing staff will make daily evaluations of the participant's general health and tolerance of the study protocol, and will insert and maintain the blood sampling IV lines following CCI SOPs. A nurse or physician will insert the continuous glucose monitor probe. A specially-trained CCI nurse and a nurse practitioner will be present throughout the intravenous glucose tolerance tests. A physician will perform an admission physical examination, will review medical and safety data on a daily basis during the inpatient study, and will be available on-call to handle any medical emergency that might arise.

A study team member (postdoctoral fellow) will meet with the participant each day to review their study experience; she will meet with the CCI staff to review their impressions of study progress; and she will review all data collection for timeliness, completeness, and accurate documentation. Moreover, Dr. Duffy and the postdoctoral fellow on the study team will also be on-call throughout the study to answer questions from CCI staff. Much of the data (PSG, waking EEG, blood sample times, saliva sample times, lights out and lights on times, subjective alertness assessments, voice data) are collected by an integrated data collection system that time stamps each piece of information, puts it into a secure database according to participant code, and is backed up automatically at least daily. Other study data (diet content, assay results, demographic information, REE results) are entered into a secure database using Standard Operating Procedures and double-checked for quality control.

Ongoing study progress will be reviewed weekly by the study team. During these meetings, the Principal Investigator and other members of the research team are present. At these meetings, protocol deviations and incomplete data are reviewed, and decisions are made regarding inclusion/exclusion and individual participant termination; such decisions (individual participant termination) that need to be considered in between the study team weekly meetings are made via conference call.

If required by the NIH, the PI will submit an annual progress report that confirms adherence to the DSMP, including a summary of any data and safety monitoring issues that occurred since the previous reporting period; and describing any changes in the research protocol or the DSMP that may or does affect risk. He will also provide the NIH with all new and continuing IRB approvals during the course of the project if requested.

How Participant Confidentiality will be Protected. We and the BWH CCI have multiple steps to protect participant confidentiality. The MGB Human Research Committee requires investigators of NIH-funded human research studies to use a special Certificate of Confidentiality Research Consent Form.

Safety Monitoring. A Safety Officer will be appointed for the study. We will select a licensed physician from outside the Division of Sleep and Circadian Disorders as the Safety Officer to avoid any potential conflict of interest. The Safety Officer's CV will be provided to the NIDDK Program Officer prior to appointment for their review.

Process by which Adverse Events (AEs), including Serious Adverse Events (SAEs and Unanticipated Problems (UPs), will be Managed and Reported. Dr. Williams will make the final determination related to any Adverse Events for reporting purposes, including the severity of any event, nature of the event, and relationship of the event to study procedures, taking information from the CCI nursing staff and any other medical personnel present during an event (such as the specially trained nurses present during the IVGTT procedures) and available data and documentation related to the event. Using this information, the PI will then follow IRB, NIH, and NIDDK guidelines for reporting the adverse event.

Timeline for Reporting Serious Adverse Events (SAEs and Unanticipated Problems (UPs). Any adverse event will be reported to the IRB as required within the time frame required to do so (typically 5 working days; serious or unexpected adverse events (AEs) will be reported immediately). The IRB in turn will work with the PI to report the serious adverse events (SAEs) to the NIH Program Officer. Any action by the IRB or the CCI that results in temporary or permanent suspension of the study will be reported immediately to the NIH Program Officer. Minor deviations and non-serious AEs are reported to the IRB annually as part of the protocol continuing review process.

Screening Laboratoy Tests and Acceptable Ranges				
	Description	**Normal Values**	**Acceptable Values**	**Comments**
Comprehenstive Metabolic Panel				**SST, 8.5mL**
Glucode (Random)	Serum Glucose	54-118mg/dl	54-150mg/dl	Non-fasting. If >150 mg/dl then repeat fasting
Glucose (Fasting)	Serum Glucose	54-118mg/dl	54-125 mg/dl	
Urea	BUN	9-25 mg/dl	6-50 mg/dl	
Creatinine	Serum Creatinine	0.7-1.3 mg/dl	0.4-1.4mg/dl	
Sodium	Serum Sodium	136-142 mmol/L	3.5-5.0 mmol/L	
Potassium	Serum Potassium	3.5-5.0 mmol/L	3.5-5.0 mmol/L	
Chloride	Serum Chloride	98-108 mmol/L	94-114 mmol/L	
Total CO2	Serum Total CO2	23-32 mmol/L	22-36 mmol/L	
Anion Gap		3-15 mmol/L	2-16 mmol/L	
ALT/GPT	Liver Enzyme	7-52 U/L	5-60 U/L	
AST/GOT	Liver Enzyme	9-30 U/L	5-40 U/L	
Alk Phos	Liver Enzyme	36-118 U/L	20-135 U/L	
Total Bili	Liver Function	0.2-1.2 mg/dl	0.0-1.9 mg/dl	1.2-1.9 mg/dl acceptable as long as other LFTs are WNL
Total Protein	Serum Protein	6.0-8.0 g/dl	5-8.5 g/dl	
Albumnin	Serum Protein	3.7-5.4 g/dl	3.5-5.9 g/dl	
Globulin	Serum Protein	2.0-4.0 g/dl	1.6-4.1 g/dl	
Calcium	Serum Calcium	8.8-10.5 mg/dl	8.7-10.5 mg/dl	
Complete Blood Count with Differential				**EDTA, 3mL**
WBC	White Blood Cells	4-10 Kl/uL	3-10.5 K/uL	
RBC	Red Blood Cells	3.9-6.0M.ul	3.0-7.0M/ul	
HCT (women)	Hematocrit	36-48 g/dl	34-48 g/dl	
HCT (men)	Hematocrit	38-49 g/dl	36.5-49 g/dl	
HGB (women)	Hemoglobin	11.5-16.4 g/dl	11.0-16.4 g/dl	
HGB (men)	Hemoglobin	12.5-16.6 g/dl	12.0-16.6 g/dl	
MCV	Blood MC volume	80-95 um3	76-100 um3	
MCH	Blood MCH	27-32 uug	24-36 uug	
MCHC	Blood Index	32-36 g/dl	30-38 g/dl	
RDW	Blood Index	10-14.5	9-15.5	
PLT	Platelets	150-450 K/ul	140-500 K/ul	
Lymph %	Lymphocyte %	18-41	10-60%	
Mono %	Monocyte %	2.5-8.5	1.8-14	
Neut %	Neutrocyte %	48-76	32-84	
EOS %	Eosinophil %	0-5	0-9	
Baso %	Basophil %	0-2.5	0-9	
Lymph #	Lymphocyte Absolute	0.8-4.1 K/ul	0.4-5.8 K/ul	
Mono #	Monocyte Absolute	0.10-0.80 /ul	0.02-1.4 K/ul	
Neut #	Neutrocyte Absolute	3.9-7.6 K/ul	2.9-9.0 K/ul	
Eos #	Eosinophil Absolute	0.0-0.5 K/ul	0.0-0.6 K/ul	
Baso #	Basophil Absolute	0.0-0.15 K/ul	0.0-0.3 K/ul	
TSH	Thyroid Stim Hormone	0.5-5.0	0.5-5.0	**SST, 3.5mL**
Urinalysis				
COLOR		clear/yellow	clear/yellow/amber/cloudy	
SP GR	Specific Gravity	1.003-1.035	1.003-1.045	
PH	pH	4.5-8.0	4.3-8.5	
PRO	Protein	Negative	1+	
KET	Ketone	Negative	1+	
BILI	Bilirubin	Negative	trace	
BLOOD	Blood	Neative	negative (Males) 1+ (Females)	
LEUK	Leukocyte	Negative	No more than one	
ES	Esterase	Negative	of these may be	
NIT	Nitrates	Negative	positive	
URO	urobilinogen	0.2-1.0	0.2-1.2	
WBC	white blood cells	0-4/hpf	<10	
RBC	red blood cells	0-2/hpf	0-2 (males) <10 (females)	
BACT	bacteria	negative	1+ (unless sq epitherial cells present)	
SQ EPI	squamous epithelial cells	variable	variable	
Drug Screen				
Comprehensive Drug Analysis (Urine)		Negative	Negative	If positive for THC, test may be repeated once
Prengancy Test (Urine)		**Negative**	**Negative**	
Nicotine Metabolite		**Negative**	**Negative**	
Alcohol/ETG		**Negative**	**Negative**	
Caffeine		**Negative**	**Negative**	
Test results that fall outside the "Acceptable" range can be repeated once to confirm findings				