NCT# 02585830

Protocol #: 15-0739
Project Title: Investigating the relationship between circadian phase and insulin resistance in obese adolescents
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I. Hypotheses and Specific Aims:

HYPOTHESIS: In obese adolescents, delayed melatonin onset and offset will correlate with insulin resistance (IR), and may be affected by sex.

SPECIFIC AIMS:

Aim 1: Establish a method to measure and model the melatonin profile, including melatonin onset and offset, as a marker of circadian rhythm in obese adolescents.

Aim 2: Develop a physiologically-based mathematical model of adolescent human sleep/wake and circadian interactions, and apply the model to investigate the mechanisms associated with published changes in sleep/wake behavior observed in adolescent sleep as well as in individual subjects.

Aim 3: Relate the timing of melatonin onset and offset to measures of insulin resistance in obese adolescents and examine sex differences among these variables.

Research Methods

Outcome Measure(s):

Primary Outcomes: Melatonin phase assessment and IR

Melatonin phase assessment: During the study visit, subjects will be admitted to the Children's Hospital Clinical research center inpatient unit at 4PM on Wednesday, Thursday or Friday. The profile of melatonin release will be assessed at the study visit using a serial saliva sampling method from 5PM on Thursday or Friday to 12PM the following day. Saliva (~2mL) will be collected in 30 or 60 minute intervals in dim light (<10 lux in the room; ~1.9 lux in the angle of gaze, confirmed by light meter) using polystyrene tubes throughout the night. Individualized melatonin sampling schedules will be determined to optimize calculation of DLMOn and DLMOff times by introducing 30 minute sampling at the times at which DLMOn and DLMOff are predicted to occur based on actigraphy-reported sleep, wake, and midsleep times[1].

Assessment of IR: During the screening visit, a 3-hour frequently sampled OGTT will be performed in the outpatient CTRC. The OGTT will take place in the AM, after a fast of at least 10 hours. A 20-gauge iv catheter will be inserted in the antecubital vein for blood sampling. After baseline samples are collected, a 3-hour OGTT with 75 mg dextrose drink will be performed. Glucose and insulin samples will be collected every 30 minutes. In addition, we will assess fasting labs to check for other components of metabolic syndrome including hemoglobin A1C for dysglycemia, ALT/AST for liver inflammation, sex hormone binding globulin as a marker of IR, cholesterol panel, CRP as a marker of inflammation, leptin as a gut hormone known to influence hepatic IR, and adiponectin, thought to influence adipose IR. Additionally, blood pressure will be taken to assess for hypertension and waist/hip ratio will be measured as a marker of metabolic disease.

Additional Measures: Questionnaires assessing additional sleep parameters, mood/behavior, and diet/activity will be completed by participants to identify variables to control for in analyses (e.g, presence of symptoms of sleep-disordered breathing) and/or utilize in exploratory analyses (e.g., relationship between sleep and diet/activity).

i. The **Morningness/Eveningness Scale for Children** [2] and the **Munich Chronotype Questionnaire for Children** [3] will be used to determine chronotype (diurnal preference).

ii. The **Cleveland Adolescent Sleepiness Questionnaire** [4], **Sleep Disturbances Scale for Children/Adolescents** [5], and the **Adolescent Sleep Hygiene Scale** [6] will be completed by participants to obtain information on behavioral sleep variables, including daytime sleepiness and sleep-promoting and inhibiting factors.
iii. The **Strengths & Difficulties Questionnaire** [7,8], an adolescent self-report tool, will be used to assess symptoms of mood and behavior problems.
iv. The **Profile of Mood States 2 Short Version**[9], an adolescent self-report measure of mood, completed on the computer.
v. The **Three Day Physical Activity Recall** (3DPAR[10]), a questionnaire asking youth to recall their physical activity levels from the previous three days, will be completed. Physical activity can directly affect insulin sensitivity, our primary outcome measure.
vi. Macronutrient intake pattern will be ascertained using the **SEARCH Food Frequency Questionnaire**, devised to include common food choices among ethnically and regionally diverse youth aged 10-19 years. The instrument is self-administered with staff support to provide instructions, answer questions, and to review the form after completion, and captures the last week of dietary intake.
vii. Psychomotor Vigilance Task[11,12], a computerized test of sustained attention with particular sensitivity to sleepiness.

viii. Description of Population to be Enrolled:

Participants: This pilot study would allow for preliminary data collection on up to 30 obese adolescents (15 male, 15 female).

Recruitment: Participants will be recruited from adolescent, obesity and community clinics.

Subject Criteria:

Inclusion criteria:

1) High school students between the age of 15-19 years (the peak developmental period for delayed circadian phase[13,14]);
2) BMI >90th percentile for age and sex;
3) Tanner stage 2 or greater.

Exclusion criteria:

1) Any medications that affect IR or sleep (e.g., metformin, hormonal contraception, stimulants, atypical antipsychotics);
2) regular use of melatonin or other sleep aids;
3) a prior diagnosis of obstructive sleep apnea, diabetes (HbA1c > 6.5), liver disease other than non-alcoholic fatty liver disease, pregnancy or breastfeeding;
4) IQ<70 or severe mental illness that may impact sleep (e.g., schizophrenia, psychotic episodes), verified through chart review;
5) teens not enrolled in a traditional high school academic program (e.g., home school students);
6) night shift employment;
7) travel across more than two time zones in the month prior to the study.

Informed Consent Plan: Appropriately qualified and informed personnel who have completed the COMIRB and HIPPAA course requirements will fully explain the study protocol and consent form to the subject and guardian verbally in the language they understand. The explanation will be conducted in a quiet environment with adequate time given for the subject and guardian to review the study procedure before the commencement of the study. Asking the subject to explain the study in their own words will assess the subject's understanding. If non-English speaking subjects are enrolled in the study, the investigators will adhere to Section 10C of the COMIRB Instructions for Clinical Investigators regarding the consent of these subjects. The qualified personnel mentioned above will then obtain written consent from the guardian and assent from the subject, co-signed on the consent form, or in subjects who are 18 years or older, direct consent. The PI will make a good faith effort to obtain both parent signatures. The subject and guardian will be provided a copy of the consent form for better understanding and record purposes.

Special Consent/Assent Plan; Consent will be obtained from all participants in the study. Following explanation, all subjects below 18 years old will co-sign the consent form in addition to the parents signing the consent form. All subjects age 18 or older will sign the standard consent form.

Subject Compensation, Incentives & Rewards

Subjects will be compensated with Greenphire debit card payments during each scheduled visit. Participants will receive $5 for the initial visit that includes signing consent and receiving the actigraphy watch. After completing the screening visit, consisting of OGTT, and questionnaires, participants will receive $50 loaded on to a debit card. Participants will receive $100 on the debit card following the overnight study visit. Compensation for all completed visits will total $155.

Study Design and Research Methods

Study Timeline: Participants will have 3 visits: an initial visit, a screening visit, and an overnight study visit. The initial visit will include informed consent and assent and receiving an actigraphy watch and instructions. The screening visit will include questionnaires, 3-hour frequently sampled oral glucose tolerance test (OGTT), and fasting labs to check for other components of metabolic syndrome. During the overnight study visit subjects will be admitted to the Children's Hospital Clinical research center inpatient unit from 4PM Thursday or Friday to 12PM the following day for salivary melatonin collection. During the entire protocol, teens will be asked to abstain from caffeine, non-steroidal anti-inflammatory drugs, marijuana, high carbohydrate meals, and alcohol.

Initial visit and sleep/wake monitoring: The initial visit will last approximately 30 minutes and will include consent and assent. Participants will be provided an actigraphy watch to monitor typical home sleep patterns[15,16]. The Spectrum sleep/wake monitor (Phillips Resperonics, Bend, OR) will be worn on the non-dominant wrist and will collect data on movements in 1-min bins. The watch will be worn for the 7 days preceding the initial visit and participants will complete a concurrent sleep diary emailed to them each morning via RedCap to allow for removal of artifacts (e.g., failure to wear the actigraphy watch during a portion of the night). If participants have not completed the sleep diary by mid-morning, a RedCap email and text message reminder will be sent. The text messages will be generic so as not to identify the participant or that they are part of a research study. If participants do not have email or computer access, they can complete a paper/pencil sleep diary.

Screening visit and sleep/wake monitoring: The screening visit will last approximately 4 hours and will ideally occur on Thursdays or Fridays in order to be consistent with the amount of accumulated sleep debt incurred over the week. Menstruating girls will be scheduled in the follicular phase when possible, as menstrual timing can influence measures of IR. Participants will receive or be mailed an actigraphy watch to wear for 7 days prior to the overnight study visit while filling out a concurrent sleep diary.

Teens will follow a self-selected sleep schedule during the pre-study week, but data collection will take place during the academic year to capture participants' typical sleep schedule. Data will not be collected during the week following daylight savings changes or in a week following travel across one or two time zones. Average sleep start and end time, sleep duration, and sleep efficiency will be measured.

Description, Risks and Justification of Procedures and Data Collection Tools:

1. Blood Sampling

Description: Blood will be drawn using a peripheral IV for HbA1c, ALT/AST, sex hormone binding globulin, a cholesterol panel, CRP, leptin, and adiponectin, and OGTT.

Risk: Minimal. Risk of pain, bruising at site of blood draw, excessive amount of blood

Minimizing Risk: The study involves sampling blood at multiple time points, thus an IV is needed to avoid multiple needle sticks. Proper sterile techniques will be used with blood draws and IV placement to decrease infection risk. EMLA cream will be used if subject desires to minimize pain of IV. The routine guidelines in our Pediatric CTRC are 2.5ml/kg for a single draw and no more than 5ml/kg over a 4 week period. For this study, the baseline labs will include 13 ml of blood and the OGTT includes 29ml of blood. Thus, our study visit is within the NIH Clinical Center guidelines of 9ml/kg in 6-8 weeks and within Children's Hospital Colorado's institutional guidelines of 5 ml/kg. We will use a minimum weight cutoff of 38 kg to remain below the most conservative pediatric CTRC blood drawing guidelines. Finally, our CTRC has a system to track other studies subjects might enroll in, and we ask during our consent process if the subject has been involved in any other studies in the past 6 weeks to avoid excessive blood drawing.

Justification: These labs are to measure for components of the metabolic syndrome, and to assess the primary study endpoint, IR. A hemoglobin A1c can be used to rule out diabetes.

2. Oral Glucose Tolerance Test (OGTT)

Description: An OGTT will be performed with multiple blood draws over 3 hours. The purpose of the OGTT is to provide a controlled oral stimulus to effect changes in lipolysis and hepatic glucose release.

Risk: The subjects rarely experience nausea within 15 min of consuming the drink, however, the amount of carbohydrate is very similar to a large soda, which is regularly consumed by this patient population.
Justification/Minimization: A standard oral challenge is needed to study insulin resistance in order to examine the relationship with sleep. Our team of investigators, CTRC pediatric research nursing staff and physicians are well experienced with the OGTT blood draw procedure. A floor nurse located on the 9th floor of CHC will be available during our inpatient visits and patients will be distracted by TV or other similar means during the OGTT, to minimize queasiness.

3: Actigraphy

Description: Each subject will wear an accelerometer for 14 days (Spectrum, by Philips Resperonics) to measure habitual sleep/wake patterns. The accelerometer will be worn on the non-dominant wrist, similar to a wristwatch.
Risk: There is no risk involved with the actigraphy device.
Justification/Minimization: Actigraphy is an effective tool for the objective measurement of sleep because they have the ability to continuously record sleep and activity data and such data can be used to estimate sleep/wake patterns. They provide more detailed information than pedometers, which only measure walking steps, and help get around the recall bias of questionnaires. We are currently using the Spectrum Actigraphy watches in adolescents in our other research studies and in routine clinical care; therefore, we are familiar with their use in this population and have the necessary computer software and interpretation skills.

4. Questionnaires

Description: Participants will complete questionnaires regarding their sleep, physical activity and eating habits, and mood and behavior.
Risk: There is a small possibility that participants will be uncomfortable answering a particular question.
Justification/Minimization: The questionnaires used in the study are widely used in both research and clinical practice, and are not of a particularly sensitive nature. Participants will be able to skip any items that they find uncomfortable. None of the items assess for suicidality.

5. Overnight stay with salivary melatonin sampling

Description: Saliva will be collected every 30-60 minutes from 5pm-noon.
Risks: Discomforts include unsettling to sleep away from home in a strange place. Providing saliva samples may make the mouth feel dry. Participants may obtain inadequate sleep.
Justification: The melatonin will provide data on the chemical signaling involved in falling asleep and waking up, as they relate to changes seen with the sleep study. Saliva is good alternative to blood sampling in pediatric populations. Short term risks are associated with inadequate sleep, including daytime sleepiness and inattention. Inadequate sleep is readily reversed with 1-2 nights of good sleep. As will be indicated on the consent form, subjects will be asked not to drive, operate heavy machinery, or engage in jobs that require a high degree of coordination, balance, or attention (e.g., roofing) for the day subsequent to the overnight visit. When reviewing the consent form, we will call attention to these prohibitions and exclude participation if they report being unable to follow them (e.g., the participant has a job that requires driving).

6. Violation of Privacy and Loss of Confidentiality

Description: These are both risks to which research participants are exposed. The possibility of these risks increases when protected health information is collected. Every effort will be made to decrease this risk by limiting access to protected health information, storing this information in a password protected database, and identifying subjects only by a unique identifier that is kept in a separate location in a locked container, traceable only by study personnel. All of the tests involve the risk of identifying asymptomatic abnormalities. The study may include risks that are unknown at this time.
Justification/Minimization: Every effort will be made to decrease the risk of loss of confidentiality by limiting access to protected health information, storing this information in a password protected database, and de-identifying study specimens.

Data Analysis Plan:

Aim 1 Analysis Plan: Demographic and clinical characteristics will be summarized by group with descriptive statistics. Prior to analysis, variables will be examined for unusual values for query, missing data, and whether distributions are non-normal, in which case a transformation may be applied.
Melatonin modeling: The timing of melatonin onset and offset will be computed according to standard procedures that have been validated in adolescents [13,17]. We will define DLMOn phase as the clock time

at which evening salivary melatonin concentrations increase and remain above a 4pg/mL threshold using linear interpolation between successive samples [17,18]. Similarly, we will define DLMOff phase as the clock time at which salivary melatonin concentrations fall below this threshold. Phase angles of entrainment will be computed as the time interval between DLMO and average actigraphic estimates of sleep variables, including bedtime, mid-sleep time, and wake time. Additional characterization of the melatonin profile, including assessment of melatonin amplitude, will also be conducted.

Aim 2 Data Modeling Plan: To investigate the relationship between sleep/wake behavior and circadian phase in obese adolescents, we will develop a physiologically-based mathematical model of sleep/wake and circadian interactions that simulates adolescent sleep patterns. Model equations will be implemented in MATLAB and solved numerically using an appropriate solver. Initially, we will fit model behavior to published characterizations of adolescent sleep/wake behavior. After establishing a representative model of adolescent sleep, we will assess the role of key model parameters in the production of key elements of adolescent sleep, and we will investigate the role of these parameters in inter-individual differences observed in sleep/wake behavior in study participants as characterized by actigraphy data.

Aim 3 Calculations and Data Analysis Plan:

Power calculation: A sample size of 30 participants will provide 80% power to detect a correlation coefficient equal to 0.47, given a 2-sided α=0.05. Because this is a pilot study, even if the null hypothesis is not rejected, the information gained regarding possible effect sizes will be useful in obtaining subsequent extramural funding for additional participants and in planning a larger study.

Calculation of insulin resistance: IR will be calculated with the Matsuda Index, a composite model for insulin sensitivity which accounts for changes in both the insulin and glucose concentrations over time [19]. The value derived from this equation is an M value of glucose uptake in mg/m^2/min, which is approximated to results that would likely have been obtained if a more invasive hyperinsulinemic-euglycemic clamp test had been performed [19]. The range of values is 0–14, with 14 being the highest level of insulin sensitivity and zero the lowest. Additional exploratory measures including B-cell function [insulinogenic index (ΔI30/ΔG30) and (ΔC30/ΔG30) and disposition index (1/IFasting × ΔI30/ΔG30) and (1/CFasting ×ΔC30/ΔG30) will also be calculated [20-22].

Relationship between circadian phase and IR: DLMOn and DMLOff will be determined from the measures of salivary melatonin concentrations as described above. Each subject will have one DMLOn time and one DLMOff time. The relationship between DLMOn and DLMOff and IR will be assessed by comparing DLMOn and DLMOff to the whole body Matsuda index using correlation and linear models. All models will have melatonin onset or offset as the outcome, and gender effects will be considered. The relationship between average actigraphy-estimated sleep duration (from sleep watch) and IR will also be tested using correlation and linear models.

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