Protocol Title: *Obstructive sleep apnea and glycemic dysregulation in adults with type 1 diabetes*

PI: *Esra Tasali, MD*

Co-I(s): *Louis Philipson, MD, PhD; Raghu Mirmira, MD, PhD*

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Study Summary

TITLE	OBSTRUCTIVE SLEEP APNEA AND GLYCEMIC DYSREGULATION IN ADULTS WITH TYPE 1 DIABETES
SHORT TITLE	*OSA and T1D*
PROTOCOL NUMBER	
METHODOLOGY	A WITHIN-SUBJECT, CROSS-OVER STUDY DESIGN IN ADULTS WITH TYPE 1 DIABETES (T1D) AND OBSTRUCTIVE SLEEP APNEA (OSA) WHO ARE USING AN INSULIN PUMP AND CONTINUOUS GLUCOSE MONITORING (CGM)
STUDY DURATION	3 MONTHS
STUDY CENTER(S)	SINGLE-CENTER
OBJECTIVES	TO DETERMINE TO WHAT EXTENT OSA CONTRIBUTES TO SUBOPTIMAL GLYCEMIC MANAGEMENT CONTROL IN ADULTS WITH T1D
NUMBER OF SUBJECTS	N=40
DIAGNOSIS AND MAIN INCLUSION CRITERIA	TYPE 1 DIABETES (T1D) AND OBSTRUCTIVE SLEEP APNEA (OSA)

List of Abbreviations

UCMC	University of Chicago Medical Center
IRB	Institutional Review Board
NIH	National Institutes of Health
OSA	Obstructive Sleep Apnea
T1D	Type 1 Diabetes
CPAP	Continuous Positive Airway Pressure
CGM	Continuous Glucose Monitor
CRC	Clinical Research Center
EHR	Electronic Health Record

1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with the applicable United States government regulations and the University of Chicago research policies and procedures.

1.1 *Background and Rationale*

Despite advances in diabetes care and technology, most adults with Type 1 diabetes (T1D) do not achieve optimal glycemic control, and consequently many are at risk for cardiovascular and other diabetes complications[1-4]. Thus, novel strategies are needed for improving glycemic control, in order to reduce cardiovascular complications and disease burden in adults with T1D.

Obstructive sleep apnea (OSA) is a common sleep disorder characterized by recurrent collapse of the upper airway during sleep, resulting in intermittent hypoxia, sleep fragmentation by transient arousals, and poor sleep quality. Continuous positive airway pressure (CPAP), applied at night, is considered the treatment of choice for persons who are diagnosed with OSA. CPAP works by delivering continuous air pressure, preventing upper airway closures during sleep, eliminating hypoxia and sleep fragmentation, and restoring sleep quality.

Current evidence suggests that ~50% of adults with T1D have OSA, and that cardiovascular and other diabetes complications are more common among those with OSA[6-12]. Moreover, cross-sectional studies suggest that adults with T1D and OSA, particularly those with moderate-to-severe OSA, have poorer glycemic control[6-8,12]. However, to what extent OSA contributes to suboptimal glycemic control in T1D is unknown. Also, the underlying mechanisms for glycemic dysregulation in T1D in the setting of OSA remain to be elucidated[5-9].

Our overall goal is to investigate the role of OSA in glycemic dysregulation in adults with T1D. We hypothesize that OSA exerts negative effects on glucose regulation in T1D, leading to suboptimal glycemic control and thus increasing cardiovascular risk in T1D. We further hypothesize that suboptimal glycemic control in adults with T1D occurs in part through OSA-induced alterations in counterregulatory hormone release and lipid metabolism, subsequently worsening glycemic control.

Adult patients with T1D and OSA who are using an insulin pump and continuous glucose monitoring (CGM) will be studied in a within-subject, cross-over design. Participants will be studied under two 14-day study conditions in randomized order with a 3-week (+/-1 week) washout period: **untreated OSA condition (Untreated Study Period)** and **treated OSA condition (CPAP Treated Study Period)**. We will perform the same assessments in each study condition.

1.2 *Objectives*

The purpose of this study is to investigate to the role of OSA in glycemic dysregulation in adults with T1D.

The primary objectives are:

- to determine to what extent OSA contributes to suboptimal glycemic management (%CV) in adults with T1D. We will compare 14-day CGM profiles (at-home) between untreated OSA vs. treated OSA conditions to determine how the presence (or absence) of OSA affects standardized CGM metrics.Glycemic variability (% coefficient of variation i.e., % CV) will be the primary outcometo determine how alterations in counterregulatory hormone release and lipid metabolism in the setting of OSA account for suboptimal glycemic management in

adults with T1D. We will compare 24-hour blood profiles of counterregulatory hormones (in-lab), between untreated OSA vs. treated OSA conditions.

2 Study Design

This is a within-subject, cross-over study in adults with T1D and OSA (n=40) who are using an insulin pump and continuous glucose monitoring (CGM). Participants will be studied under two 14-day study conditions in randomized order with a 3-week (+/-1 week) washout: **untreated OSA condition (Untreated Study Period)** and **treated OSA (CPAP) condition (CPAP Treated Study Period)**. We will perform the same assessments in each study condition.

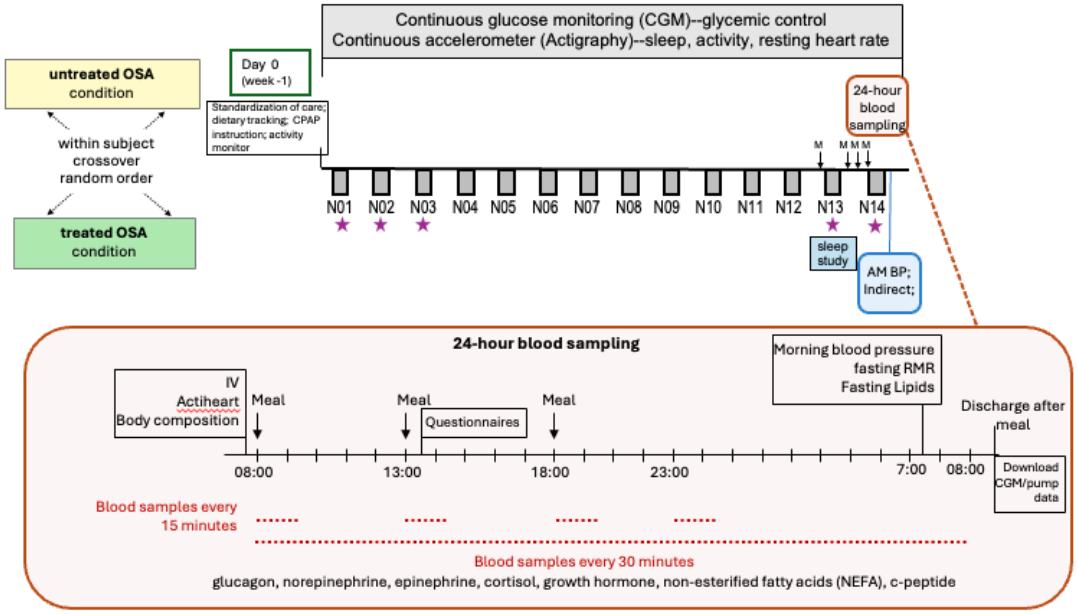

Fig 4. Study protocol. Within subject, crossover design with two 14-day study conditions in a randomized order with a 4-week washout period: **untreated OSA** and **treated OSA** (receiving all-night continuous positive airway pressure, CPAP).★ Participants spend nights 1-3, 13 in-lab (bedtimes 23:00h to 7:00h) and engage in their daily routine activities during the day. Day 14 is spent in-lab (bedtime 23:00h to 7:00h) for 24-hr blood sampling. Identical, isocaloric diabetic meals (M) are served at 08:00h,13:00h and 18:00h with blood samples every 15min (for 90min) at 23:00h and after each meal. Sleep continuously monitored by Actigraphy. Daily weights taken at-home. Overnight sleep study (polysomnography) will be performed on night N13.

2.1 *General Description*

Research Location: This research will be conducted in the research units located at the Clinical Research Center (CRC) on W5 floor and UChicago Sleep Center on W4 floor of the Mitchell building at the University of Chicago. This research will be conducted mainly in the UChicago Sleep Center, except for some cases where the 24-hour sampling may take place in Clinical Research Center (CRC). This will depend on nursing availability.

Informed Consent: Subjects will be consented in clinic. Written informed consent will be obtained after a member of the research team has explained all details of the study, and the subject has received satisfactory answers to all of his/her questions. After informed consent, the subjects will undergo screening procedures. Details of all screening procedures are provided below under section 5. Study Procedures.

Screening Procedures: After informed consent, the subjects will undergo a single blood test (Visit 1) and home sleep apnea test (Visit 2). Details of all screening procedures are provided below under section 6. Study Schedule.

Study Procedures: After screening, eligible subjects will be studied using a cross-over design under two conditions, **untreated OSA (Untreated Study Period)** and **treated OSA (CPAP Treated Study Period)**, that will be done in random order with a 3-week (+/-1 week) washout period. Prior to the study, sleep condition order assignments will be prepared by the study statistician using block randomization, stratified by sex.[10] Each study condition will be over a 14 day period. Over the 14-day period subjects will be asked to home monitor (wrist activity monitor & food logging, digital weight scale) and complete a daily questionnaire about their last night's sleep. The first three overnights (Days 1-3) will be spent in lab from 21:00h-7:00h. Days 4-12 will be spent at home, and the participants will continue to home monitor their activity/sleep and nutrition. On Day 13 subjects will complete an overnight in lab sleep study. Upon waking (Day 14) subject will then complete 24-hour blood sampling in the research unit. Details of all study procedures are provided below under section 5. Study Procedures and 6. Study Schedule.

2.2 *Number of Subjects*

A total of 40 subjects will be enrolled (randomized) over 5-year period to achieve a final sample of 34 subjects completing the entire study after dropouts.

2.3 *Duration of Participation*

The duration of participation for each subject would take about 3 months, including 1 screenings visit and 4 study visits with 3-week (+/- 1 week) washout periods between study visits. The overall recruitment and completion of the study would take about 5 years.

2.4 *Study Endpoints*

Study endpoints will be:
14-day CGM profiles (standardized CGM metrics) to assess glycemic management including mean glucose, glycemic management indicator (GMI), glycemic variability ((% coefficient of variation i.e., %CV), and time in ranges (time below, within, and above the established glycemic targets). Glycemic variability (% CV) will be the primary outcome.
24-hour blood profiles (glucagon, adrenaline, noradrenaline, cortisol, growth hormone, free fatty acids, glycerol) measured under controlled in-lab conditions with standardized meals. Additional biological and behavioral factors that may influence glycemic management: sleep stages by polysomnography (e.g., slow wave sleep), insulin dose (from pumps), self-reported sleepiness and subjective sleep quality (Morning Sleep Diary), diabetes self-management behaviors (i.e., self-monitoring of diet using 'Lose It' a smartphone app, objective physical activity by accelerometry, validated questionnaires), resting metabolic rate (indirect calorimetry) and body composition (InBody 570 and daily weights on digital scale). We will also measure resting heart rate (Actiheart, wearable monitor), and fasting lipids to determine how presence (or absence) of OSA impact these intermediate markers of cardiovascular risk in adults with T1D.

3 Subject Selection and Withdrawal

3.1 *Subject identification and recruitment*

Subjects will be recruited in-person during routine clinic appointments in the Diabetes clinic and Sleep clinic.

3.2 *Inclusion*

- Age: 18 to 50 years old
- Type 1 Diabetes (duration 3-20 years) on insulin pump therapy and using a CGM device with an GMI (glucose management indicator) between 5.5 and 8.5% with hemoglobin in the normal range at screening
- Moderate to severe OSA by home sleep apnea test

3.3 *Exclusion*

- Regular and adherent CPAP use per clinical guidelines
- Requiring oxygen or advanced positive airway pressure modalities during sleep
- Having a 'fall-asleep' or 'near miss' accident in the past 6-months
- Shift work
- Severe hypoglycemia (≥1 episode in the past 3 months or diagnosis of hypoglycemic unawareness)
- ≥1 trip to emergency room for poor glucose management in the past 6 months
- Proliferative retinopathy
- Fasting triglycerides >400mg/dL,
- Liver transaminases >2 times upper limit of normal,
- Renal transplantation or serum creatinine >1.5 mg/dL
- Anemia (hemoglobin <13.0g/dL in men or <11.6g/dL in women)
- Acute coronary syndrome or stroke past 6 months
- Severe hypertension (blood pressure>180/105 mmHg)
- Any other significant health condition: unstable angina, heart failure requiring hospitalization in the past 6 months, significant heart block or arrhythmias, NYHA Class>2, pulmonary disease with dependence on oxygen or daily use of bronchodilators, active or chronic infection, thyroid disease and other endocrine disorders (e.g. Cushing syndrome, acromegaly)
- Recent major surgery
- Major psychiatric disorder
- Subjects will also be excluded if taking medications that can confound metabolic assessments including systemic glucocorticoids, antipsychotics, thiazide diuretics, beta-blockers, daily use of aminophylline or theophylline, or use of any immunosuppressant.
- Currently pregnant or trying to get pregnant or nursing
- Smoking, alcohol or illegal drug abuse

3.4 *Early Withdrawal of Subjects*

<u>When and How to Withdraw Subjects</u>
Subjects must be withdrawn from the study for any of the following reasons:

- Withdrawal of informed consent

- Pregnancy
- If the investigator concludes that it would be in the subject's best interest for any reason

Subjects may voluntarily withdraw from the study for any reason at any time. If withdrawal occurs for any reason, the investigator will document the reason for withdrawal in the source notes.

Data Collection and Follow-up for Withdrawn Subjects
Subjects who withdrew after enrollment will be replaced to complete the study.

4 Study Drug/Device

4.1 *Description*

Subjects will complete both study periods (conditions), **Untreated Study Period** and **CPAP Treated Study Period,** in randomized order with a 3-week (+/- 1 week) washout period.

4.2 *CPAP*

During the **CPAP Treated Study Period**, all-night CPAP will be applied with bedtimes 23:00h to 7:00h. Days 1-3 will be in-lab under supervision for 3 nights to achieve complete resolution of OSA using optimal pressure and mask settings. On days 1-3 while sleeping in the research unit the overnight technician will be able to provide immediate help to the subjects for adjusting their mask and gain familiarity with the CPAP machine. Days 4-12 subjects will sleep at home with their CPAP machine and to follow regular bed and wake-up times 23:00h to 7:00h.

CPAP treatment: CPAP masks can cause skin irritation from the mask and nose/mouth dryness.
Risk Reduction: Our sleep technicians have extensive experience with CPAP therapy and these risks will be minimized with appropriate mask choice and adjustment of device humidity settings.

Untreated severe sleep apnea: Untreated severe sleep apnea may carry risk of excessive daytime sleepiness and drowsy driving and car accidents.
Risk Reduction: We will exclude subjects with high-risk occupation for drowsy driving (e.g. commercial truck driver or airline pilot). All subjects will be questioned regarding 'fall-asleep' accidents or near miss accidents and such events will be notified to the DSMB before deciding on whether they should continue the study. The duration for study participation will be 3 months, and in large CPAP trials lasting from 6 months to more than 3 years (e.g. APPLES, SAVE trials), no increase in adverse events (cardiovascular or driving accident) were observed in patients with moderate or severe sleep apnea who were randomized to "non-treatment" arms, indicating overall safety of this protocol. All participants will be provided with a report summarizing the results of their sleep evaluations, a brochure on sleep apnea and its consequences, and information on available sleep centers to obtain further care. The standard clinical time from appointment to obtaining an at-home CPAP machine is anywhere from 3-6 months and subjects can be completing this process during the study.

5 Study Procedures

5.1 *Continuous Glucose Monitor (CGM)*

Glycemic management will be assessed over 14 days by CGM. Participants will have their own CGM device as a part of their standard clinical care. Commonly used standalone FDA-approved CGMs include several models of the Libre, including Libre 14-day, Libre 2, Libre 3, Libre 2 Plus, Libre 3 Plus; Dexcom G6 and G7, Eversense E3 and 365 and Medtronic Guardian 3 and 4. Using CGM profiles, we will derive standardized CGM metrics[14] that are used in clinical care to assess glycemic management. The study team will request access to the data from the CGM directly from the participant. The participant will be asked to share their CGM data with the research team (clinical research coordinators, clinical research nurse) using the connection methods that are available for their device in order to have data for the dates of the study.

5.2 *Insulin Pump*

Participants will have their own insulin pump as a part of their standard clinical care. All insulin pumps are readily interrogated for a variety of daily and cumulative statistical measures, including bolus/basal ratio, percentages, and readily show boluses for each meal, changes in basal rates, how much insulin is delivered by the user and how much insulin is delivered by the pump. The study team will request access to the data from the insulin pump directly from the participant. The participant will be asked to give the research team (clinical research coordinators, clinical research nurse) access through the product-specific web-based portal in order to have data for the dates of the study.

5.3 *Dietary Tracking App 'Lose It'*

Diet will be captured using a standard, commercial smartphone app named "Lose it." Participants will be instructed to record their daily dietary intake into the app. While marketed as a weight loss tool, it is used in this study because it is easy to use, tracks macronutrients, and data can be accessible to the research team. Importantly, the app has a scanner feature to track package barcodes and has integration with cell phone cameras to allow logging of food that might not have bar codes. Participants will be asked to download the data from the 14 days and give to the study team. The data includes date, type of food, serving size and macronutrients.

5.4 *Activity Monitor*

Sleep, physical activity and heart rate will be continuously and objectively assessed by an 3-axis accelerosensor (Actigraph LEAP) worn on subject's wrist during each 14-day study condition and 1 week preceding each study condition to also capture habitual patterns at home.

5.5 *Digital Weight Scale*

Body weight and composition will be measured by a take-home digital weight scale (Whitings BodyPro 2). During each 14-day study condition subject will be asked to step on the scale within 15 minutes or wake and after they void.

5.6 *Heart Monitor*

On Day 14 during the 24-hr blood sampling we will also collect data using a heart monitor, the Actiheart ECG device. The Actiheart is a very small, waterproof, ambulatory device consisting of a single-lead ECG and a tri-axis accelerometer to monitor heart rate, and physical activity and posture. It is worn on the chest (under the clothing) using standard disposable ECG electrodes. This data will allow us to continuously assess the resting heart rate and heart rate variability, which are strong prognostic markers of cardiovascular health.

5.7 *Body Composition*

To measure body composition we will use the InBody 570 Body Composition Analyzer located in the Clinical Research Center. This will be done in the morning, in a fasted state in a hospital gown to measure fat mass and fat-free mass.

5.8 *Indirect Calorimetry*

Resting energy expenditure will be measured for 20 min by indirect calorimetry in fasted state.

5.9 *Overnight Sleep Study*

Full polysomnography (overnight recording of sleep including EEG, oxygen saturation, respiration) overnight in the laboratory will be done to assess sleep on Day 13 of both the Untreated and CPAP Treated Study Periods.

5.10 *Meals*

All meals will be prepared under the supervision of a Registered Dietitian at UChicago Clinical Research Center's Metabolic Kitchen. Caloric needs will be calculated for each participant based on indirect calorimetry. A diabetic breakfast will be provided every morning after overnight stays. In the evening on Day 13 and 14, the participants will receive an in-lab weighed diabetic dinner at 19:00h and an evening snack before bed. On Day 14 while subjects will be in lab for 24-hr blood sampling, they will be provided isocaloric diabetic meals at 08:00h,13:00h and 18:00h. Breakfast will be provided upon waking and after blood sampling is complete (08:00h). Snacks/juice will be available at any time to prevent and/or in response to hypoglycemia. All meals will be consumed completely within 20 min.

5.11 *Questionnaires*

Upon awakening each morning, subjective sleep quality and daytime sleepiness will be assessed by the Morning Sleep Diary. This is a combination of a validated a tool, Karolinska Sleep Diary[15,16] and questions on any sleep disturbances due to CPAP mask/pressure, alarms from diabetes technologies (i.e., insulin pumps and CGM), and other factors related to lab/home environment that may influence subjects' sleep. We will assess quality of life by Diabetes Quality of Life Measure[17], diabetes self-care activities by Diabetes Self-management Questionnaire[18], hypoglycemia confidence by Hypoglycemia Confidence Scale[19], and treatment satisfaction by Diabetes Treatment Satisfaction Questionnaire[20] on Day 1 and Day 14.

5.12 *Blinding*

Prior to the study, sleep condition order assignments will be prepared by the study statistician using block randomization, stratified by sex.[13] The randomization order and sleep condition will be blinded to the resercah personnel who is responsible for data entry and data download.

6 Study Schedule

SEE SCHEMA APPENDIX B

6.1 *Visit 1- Screening*

This visit will be screening step 1, this visit will take up to 3 hours. Height and weight will be measured. Subjects will undergo screening fasted single blood draw (complete blood count, Lipid panel, metabolic panel, c-peptide level). Subjects will also undergo vitals, demographics, urine pregnancy test for women of childbearing potential and medical history and record of family history of any diabetes. Study nurse will document any diabetes complications based on subject medical health record. Subjects will undergo 20-min indirect calorimetry. This will allow subjects time to become familiar with the plastic hood that will be used during indirect calorimetry testing during study to ensure they tolerate this testing.

Eligible subjects will then be given an at home sleep test (WatchPAT One) to diagnose OSA (moderate to severe OSA). Subject will sleep with WatchPAT One device on for one night. WatchPAT One includes a snore/body position sensor worn as a sticker on the chest, small monitoring device worn on the wrist, and a finger probe worn on the index finger. Device is pre-programmed to record and is put on and worn by subject overnight at home then can be disposed of the next day.

6.2 *Randomization*

Randomization will occur after screening (Visit 1) is complete and subjects are determined to be eligible. The Untreated Study Period and CPAP Treated Study Period may happen in any order based on randomization assignment. The first study visit will occur within 4 weeks of Visit 2. If the timing exceeds 4 weeks then a single blood draw will be performed to assess no significant health changes.

6.3 *Visit 2 – CPAP Titration*

Subject will undergo an overnight sleep test (CPAP titration) by laboratory polysomnography to identify optimal CPAP pressure settings. Subjects will be asked to sleep in the laboratory with 8-hour bedtimes from 23:00h to 7:00h.

6.4 *Untreated Study Period*

6.4.1 Untreated Day 0 (-1 week (-3days))

Untreated Study Period Day 0 will be a 3-hr visit to the lab. Subjects will meet with our certified diabetes care and education specialist to receive standard diabetes education, and review and optimize their pump/CGM use and to ensure that they are using the device and alarm settings properly and optimally. Alarm systems will be set to actionable levels to minimize sleep disruption. Participants

will have their own CGM device and insulin pump as a part of their standard clinical care. On this day participants will be asked to download the 'Lose it' app on their phones, if they don't already have it, and asked to begin tracking their dietary intake. Subjects will receive the wrist activity monitor (Actigraph LEAP).

6.4.2 Untreated Days 1-14

Subjects will be asked to wear wrist activity monitor and record their dietary intake into smartphone app 'Lose it' for the 14 days of study and for the week before study. During the 14 day study condition subjects will be asked take their daily weights using the at-home digital scale. Subjects will spend the first three overnights (Days 1-3) in the laboratory with 8-hour bedtimes from 23:00h to 7:00h, they will be asked to arrive at 21:00h. After the overnights (Days 1-3) the subject will be asked to step on the InBody 570 Body Composition Analyzer before leaving the laboratory. Each morning, upon wake, subjects will complete the Morning Sleep Diary online (RedCap) about their last night's sleep. A diabetic breakfast will be provided every morning after overnight stays. Participants will leave the laboratory each morning after breakfast to engage in their daily routine activities. On days 4-12 subjects will be sleeping at home and sleep will be monitored at home by actigraphy.

On day 13 subjects will arrive in the research unit at 20:00h and will receive an in-lab weighed diabetic dinner at 20:00h. Subjects will stay in the research unit and sleep will be recorded by polysomnography with bedtimes 23:00h to 7:00h. The technician during calibration will ask the subject to complete a series of breathing maneuvers to evaluate neuropathy[26]. Upon waking on Day 14, the clinical research nurse will then place a sterile intravenous catheter in the non-dominant forearm for venous blood sampling and place the Actiheart on subject. Body composition will also be measured on the Bio570 Analyzer. Blood samples will be collected beginning at 8:00h. Blood samples will be collected every 15 min for 90 min after each meal (detecting meal responses) and after bedtime (detecting major growth hormone pulse), and every 30 min at all other times until the next day 8:00h. During waking hours, blood samples will be collected at bedside while participants remain semi-recumbent at a 30^{o} angle to avoid any influence of body position on hormone release (e.g., norepinephrine). During sleep hours, participants will be lying in bed and catheter line will be extended and fed through a port in the wall, allowing for disturbance-free blood drawing from an adjacent room. On Day 14 (i.e., 24-hour sampling day), participants will receive 3 identical isocaloric diabetic meals served at 08:00h, 13:00h, and 18:00h with snacks/juice available at any time to prevent and/or in response to hypoglycemia. Participants will be monitored for any signs and symptoms of hypo/hyperglycemia by the research staff.

On day 14 the morning blood pressure will be measured and a 20 min indirect calorimetry in fasted state will be performed. Upon the conclusion of blood pressure the research team will request access from the subject to the past 13 days of data from the subjects CGM and insulin pump.

After each night, subjects will be asked to complete the Morning Sleep Diary in the morning. On Day 1 and Day 14 subjects will complete questionnaires as described in 5.9. These will take approximately 20 minutes to complete. All questionnaires will be completed on REDCap.

6.5 *Washout Period*

During the 3 week washout period (+/- 1 week), subjects will be instructed to continue their typical daily routines. No study related activites will happen during this time.

6.6 *CPAP Treated Study Period*

6.6.1 Treated Day 0 (-1 week (-3days))

Treated Day 0 will be a 3-hr visit to the lab. All procedures described above under Untreated Day 0 will be repeated. In addition, subjects will also be fit for a CPAP mask and receive instruction on sleep apnea and the CPAP machine education to help them feel comfortable using at home.

6.6.2 Treated Days 1-14

The procedures involved in the CPAP Treated Study Period are the same as those during the Untreated Study Period, with the addition of the CPAP treatment. For the CPAP treatment, all-night CPAP intervention (using each participant's optimal pressure settings) will be applied under continuous supervision in-lab for the first three overnights (Days 1-3) to achieve complete resolution of OSA. While sleeping in the research unit, the overnight technician can help the subject adjust the mask and gain familiarity with the CPAP machine. Days 4-12 subjects will be sleeping at home with a CPAP machine (set at their optimal pressure settings). Successful treatment of OSA will also be verified from CPAP machine remotely and mask/pressure adjustment will be made as needed.

Sleep will be monitored at home by actigraphy on Days 1-14.

7 Statistical Plan and Considerations

We propose to study n=40 individuals with T1D and OSA using a within-subject design. We will obtain the same metabolic and cardiovascular assessments under two 14-day study conditions in a randomized order with a 3-week washout period: untreated OSA condition vs. treated OSA condition (receiving CPAP intervention). Thus, this will be a 2-period cross-over study that is uniform within periods (i.e., half of the participants will do untreated condition first then treated condition, the other half will do treated condition first then untreated condition). Prior to study start, condition order assignments will be prepared by the study statistician using stratified, block randomization. Sex will be the only stratification factor. This randomization schedule will then be uploaded to REDCap.

7.1 *Primary analyses*

The primary analytic approach will be to use a mixed-effects linear regression model to estimate the effects of treatment condition on outcomes along with inclusion of period, sequence, and period by treatment condition interaction and a random subject effect to account for the repeated measurements for each subject. Mean (standard deviation [SD]) values for all continuous variables, and frequency counts and percentages for all categorical variables will be used for descriptive analyses, overall and separately by treatment condition. Normality will be tested, using the Shapiro-Wilk test and Q-Q plots, for all continuous variables.

If there is evidence of non-normality, then a transformation such as the natural logarithm will be used. We hypothesize that OSA exerts negative effects on glucose regulation leading to suboptimal glycemic management and thus increasing cardiovascular risk in T1D. We further hypothesize that glycemic dysregulation in adults with T1D and OSA occurs in part through dysregulated counterregulatory hormone release and lipid metabolism, subsequently worsening glycemic management. To test these hypotheses, we will determine: to what extent OSA contributes to suboptimal glycemic management in adults with T1D (Aim 1) and to determine how alterations in counterregulatory hormone release and lipid metabolism in the setting of OSA account for suboptimal glycemic management in adults with T1D. (Aim 2).
In Aim 1, the untreated OSA vs. treated OSA condition contrast in the mixed-effects linear regression model (described above) will be of primary interest. We will compare 14-day CGM profiles between untreated OSA vs. treated OSA conditions to determine how the presence (or absence) of OSA affects glycemic management. The outcome variables (e.g., glycemic variability, time in range of glucose) will be derived from standardized CGM metrics that are used in clinical care to assess glycemic management.[14] The number of days CGM worn, percentage of time CGM is active, mean glucose, glycemic management indicator (GMI), glycemic variability (% coefficient of variation, %CV), and time in ranges (time below, within, and above the established glycemic targets) will be derived based on consensus recommendations.[14] Glycemic variability (%CV) will be the primary outcome. The glycemic variability metric will be calculated for each participant for each condition using three approaches: (1) using average data across all days, (2) using data only from day 13 (i.e., the final day prior to 24-hour sampling), and (3) using data from individual days for each subject with inclusion of day number as a factor in the mixed-effects model. Each CGM metric will be calculated using data from the 24-hour, daytime, or nighttime periods, separately.

In Aim 2, We will compare 24-hour hormonal/metabolic data between untreated OSA vs. treated OSA conditions and determine their relative contributions to glycemic management in T1D. These analyses will provide novel mechanistic insights into how OSA may contribute to glycemic dysregulation in T1D. The role of these potential mediators contributing to glycemic dysregulation will be assessed comparing untreated OSA vs. treated OSA conditions. The summary variables for each mediator (e.g., average values [24-h, daytime, nighttime], meal responses [area under the curve] from hormonal profiles) will be calculated, as previously described.[11,12,21,22] First, we will determine the magnitude of the changes in the mediator variables (untreated – treated) using paired t-tests. We will then examine the correlation between the differences (untreated – treated) in our glycemic variables (e.g., glycemic variability [%CV]) and the differences in the mediator variables. With a sample size of n=40 and a two-sided alpha level of 0.05, we will have 80%, 85%, and 90% power to detect correlation coefficients as small as 0.43, 0.46, and 0.49, respectively. A more formal estimation and testing of mediation (i.e. mechanisms that produce the effect) and moderation (i.e. factors that affect the magnitude of the effect) will also be performed using the linear regression framework, as described by Judd et al.[52] In brief, a within-subject analysis with a varying X (for example Glucagon) that can serve as both a moderator and a mediator will be conducted by assessing whether the magnitude of the treatment difference in Y (for glycemic variability, CV*) depends

on two different linear functions of the Xs: their sum and their difference as follows: (CV*treated – CV*untreated)= b0 + b1*(Glucagontreated + Glucagonuntreated) + b2*(Glucagontreated – Glucagontreated) + e, where e is an error term. Moderation will be indicated if the treatment difference in CV* depends on the sum of the two Glucagon measurements (i.e., the b1 coefficient is significant). Mediation will be indicated if the treatment difference in CV* depends on the difference between the two Glucagon measurements (i.e., the b2 coefficient is significant), assuming an overall treatment effect in both Glucagon and CV*.

7.2 *Secondary analyses*

We will calculate the untreated OSA vs. treated OSA condition contrast to examine the impact of OSA on additional factors that may influence glycemic management (e.g., insulin dose, physical activity) and determine to whether OSA impacts intermediate markers of cardiovascular risk (e.g., 24-hour ambulatory blood pressure). We will also examine the impact of variables (by including them in models as covariates) that could change between study conditions such as BMI, body composition, menstrual cycle, sleep duration, sleep stages (e.g., slow wave sleep), circadian phase53, and OSA indices (e.g., AHI, oxygen desaturation index [ODI], arousal index). We will also assess the impact of those variables that are stable over the study period (e.g., age, sex, race/ethnicity) on the magnitude of change in measured outcome variables. Models will also be fit separately to examine whether the treatment effects are significantly different by baseline OSA status (e.g., mild vs. severe OSA; hypoxic vs. non-hypoxic OSA) with the inclusion of the appropriate interaction terms in the mixed model.

7.3 *Missing data*

To account for missing data, multiple imputation using chained equations[23-25] will be considered. To further address missing data (e.g., CGM data), a sensitivity analysis only including subjects with a given level of data completeness (70% of data from 14-day CGM per recommendations[14]) will be conducted.

8 Risks and Benefits

Blood sampling/Venous Catheter: There is minimal discomfort associated with venipuncture and slight risk of bruising, or rarely fainting and phlebitis with catheter placement. The hand or arm (in which an IV catheter has been inserted) will be wrapped in a heating pad that circulates warm water to facilitate frequent blood sampling during metabolic testing. With prolonged exposure to continuous heat, there is a potential risk of local skin irritation or a minor burn.
Risk Reduction: The samples are collected using aseptic technique in designated areas of the research unit where facilities are available should untoward reactions occur. If phlebitis occurs, it will be treated conservatively with heat and when appropriate, with antibiotics. The catheter will be cared for by experienced research nurses. The amount of blood withdrawn will be in accordance with the University of Chicago IRB regulations. To minimize risk, subjects with anemia (hemoglobin <13.0g/dL in men or <11.6g/dL in women) will be excluded.

Hyper/Hypoglycemia: During the study, subjects may have high or low blood sugars, which could cause a range of symptoms including possible flushing,

sweating, dizziness, confusion, racing heart, headache, blurry vision, thirst or excessive urination.
Risk Reduction: The research center is equipped with rescue medications and equipment in the event that a subject would experience an adverse event during the test. Experienced nurses and physicians monitor all subjects during their participation in the study. In the event of excessive or symptomatic hypoglycemia, glucose may be given either through the IV or orally, as needed. If subject is experiencing symptoms or glucose approaches or is less than 70mg/dL, the hypoglycemia will be treated by consuming 15 g of rapid acting glucose. Examples of 15 g of rapid acting glucose:

- 1/2C (4oz) juice
- 1 C (8oz) milk
- 4 pieces hard candy
- 4 glucose tabs

The research nurse will monitor for 15 minutes then recheck blood sugar. If blood sugar is still less than 70, the steps above would be repeated until resolved.

InBody 570 Anaylzer: Body Composition scan to measure fat free mass using electrical pulses.
Risk Reduction: The amount of electrical pulses given during the InBody scan is very low. Subjects with artificial electrical implants, like a defibrillator or pacemaker will not be included in study, but a safety questionnaire will be asked before every scan.

Indirect calorimetry: This is a non-invasive measure of resting energy expenditure in which a clear plastic hood is placed over the head and shoulders for a period of time, during which the subject is asked to relax and remain still. The hood may cause feelings of claustrophobia.
Risk Reduction: A research staff will be present throughout indirect calorimetry testing to ensure full comfort of the subjects during the test. To minimize risk, the subjects with claustrophobia will be excluded at screening.

Untreated severe sleep apnea: Untreated severe sleep apnea may carry risk of excessive daytime sleepiness and drowsy driving and car accidents.
Risk Reduction: We will exclude subjects with high-risk occupation for drowsy driving (e.g. commercial truck driver or airline pilot). All subjects will be questioned regarding 'fall-asleep' accidents or near miss accidents and such events will be notified to the DSMB before deciding on whether they should continue the study. The duration for study participation will be 3 months, and in large CPAP trials lasting from 6 months to more than 3 years (e.g. APPLES, SAVE trials), no increase in adverse events (cardiovascular or driving accident) were observed in patients with moderate or severe sleep apnea who were randomized to "non-treatment" arms, indicating overall safety of this protocol. Upon completion of the study, all participants will be provided with a report summarizing the results of their sleep evaluations, a brochure on sleep apnea and its consequences, and information on available sleep centers to obtain further care. The standard clinical time from appointment to obtaining an at-home CPAP machine is anywhere from 3-6 months and subjects can be completing this process during the study.

CPAP treatment: CPAP masks can cause skin irritation from the mask and nose/mouth dryness.
Risk Reduction: Our sleep technicians have extensive experience with CPAP therapy and these risks will be minimized with appropriate mask choice and adjustment of device humidity settings.

Confidentiality: It is possible that the information that we collect may be accessible to individuals that are not part of the research team.
Risk Reduction: Data will be stored in a locked office and on a password protected computer at the University of Chicago. The CGM data will be accessed only by the research team for the two 14 days subjects are in the study.

Alternative to participating: Subjects can decide not to participate in this study, this will not affect their standard of care. During the study subjects' standard of care will continue. If there are any changes (i.e. medication, insulin dosage etc..) the study team will document.

9 Safety and Adverse Events

9.1 *Definitions*

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)
Any unanticipated problem or adverse event that meets the following three criteria:

- Serious: Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**
- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, **AND**
- Related: A problem or event is "related" if it is possibly related to the research procedures.

Adverse Event
An untoward or undesirable experience associated with the use of a medical product (i.e. drug, device, biologic) in a patient or research subject.

Serious Adverse Event

Adverse events are classified as serious or non-serious. Serious problems/events can be well defined and include;

- death
- life threatening adverse experience
- hospitalization
- inpatient, new, or prolonged; disability/incapacity
- persistent or significant disability or incapacity
- birth defect/anomaly

and/or per protocol may be problems/events that in the opinion of the sponsor-investigator may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

All adverse events that do not meet any of the criteria for serious, should be regarded as **non-serious adverse events**.

Adverse Event Reporting Period

Any adverse event occurring during the course of the study period will be reported.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event

All unresolved adverse events should be followed by the sponsor-investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the sponsor-investigator should instruct each subject to report, to the sponsor-investigator, any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances: *(describe any*

hospitalization situations which do not necessitate reporting as adverse events. Consider…

- *Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should **not** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.*
- *Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.*
- *Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.*

9.2 *Recording of Adverse Events*

At each contact with the subject, the study team must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event section of the case report form (CRF) or in a separate adverse event worksheet. All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results should be recorded in the source document.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period must be followed up, to determine the final outcome. Any serious adverse event that occurs during the Adverse Event Reporting Period and is considered to be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

9.3 *Reporting of Serious Adverse Events and Unanticipated Problems*

When an adverse event has been identified, the study team will take appropriated action necessary to protect the study participant and then complete the Study Adverse Event Worksheet and log. The principal investigator will evaluate the event and determine the necessary follow-up and reporting required.

9.4 *Reporting: notifying the University of Chicago IRB*

The principal investigator will report to the University of Chicago IRB any UPIRTSOs and Non-UPIRTSOs according to the University of Chicago IRB Policy and Procedures.

According to the University of Chicago IRB Policy any serious adverse event (SAE) which the Principal Investigator has determined to be a UPIRTSO would be reported to the IRB as soon as possible but no later than 5 working days after the investigator first learns of the problem/event. The following information to collect when developing any forms for documentation of adverse events.

Information collected on the adverse event worksheet (and entered in the research database):

- Subject's name:
- Medical record number:
- Disease/histology (if applicable):
- The date the adverse event occurred:
- Description of the adverse event:
- Relationship of the adverse event to the research (drug, procedure, or intervention*):
- If the adverse event was expected:
- The severity of the adverse event: (use a table to define severity scale 1-5**)
- If any intervention was necessary:
- Resolution: (was the incident resolved spontaneously, or after discontinuing treatment)
- Date of Resolution:

The principal-investigator will review all adverse event reports to determine if specific reports need to be made to the IRB. For this protocol, only directly related SAEs/UPIRTSOs will be reported to the IRB.

9.5 *Stopping Rules*

The entire study may be discontinued at the discretion of the PI based on the occurrence of the following:

- Adverse effects that seriously impact the risk-benefit ratio have been observed
- Medical or ethical reasons affecting the continued performance of the study
- Difficulties in recruitment of subjects
- Any new information becomes available during the trial that necessitates stopping the trial
- Other situations that may warrant stopping the trial

Subjects will be informed that they are free to withdraw from the study at any time for any reason. The Principal Investigator (PI) may remove a subject from the study if, in the PI's opinion, it is not in the best interest of the subject to continue the study. Subjects may be discontinued due to a change in compliance with an inclusion/exclusion criterion that is clinically relevant and affects subject safety, occurrence of AEs, occurrence of pregnancy, or administration of non-permitted concomitant medication that might affect subject safety or study assessments/objectives. In case of premature discontinuation of study participation, efforts will be made to perform end of study/follow up assessments. The date the subject is withdrawn from the study and the reason for discontinuation will be recorded on the subject's Case Report Form (CRF). All withdrawn subjects will be followed until resolution of any AEs or until any unresolved AEs are judged by the PI to have stabilized.

9.6 *Data and Safety Monitoring Plan*

The PI ensures that informed consent is obtained prior to performing any research procedures, that all subjects meet eligibility criteria, and that the entire study protocol is conducted according to the IRB-approved research plan.

Additionally, the Data Safety Monitoring Board (DSMB) will monitor the study. DSMB members will be independent of the investigators and comprised of specialists in type

1 diabetes, sleep medicine and metabolic studies who can oversee the study and offer guidance to the PI. The DSMB will have three voting members. The DSMB will convene in person or virtual every 6 months. will review adverse event data, other safety data, quality and completeness of study data, and enrollment data at each meeting to ensure proper study conduct. It will also conduct an ongoing assessment of clinical equipoise in the study. Study personnel should provide any new literature particularly pertinent to the study, along with their recommendation as to whether it affects the study conduct or design. The DSMB will review the informed consent form when it reviews the protocol. The DSMB will review the consent periodically and/or as needed and consider whether the consent form requires revision in light of any new findings or amendments. Based on an overall assessment of risk and review of the data, the Board will make recommendations, including whether the study should continue and/or be modified.

In addition to regular meetings, it may be necessary to convene the DSMB urgently on an ad hoc basis to discuss new data or other information that raises questions about equipoise, safety, or anything else in the study.

The expertise of the attending members should be appropriate for the agenda of the meeting. It's expected that all DSMB members will attend every meeting, but, this may not always be possible. Therefore, the DSMB may establish a quorum for voting. A quorum is 2 members (usually half the members plus 1). The Board Chair and in most instances the Biostatistician, must be present at all meetings. All standing Monitoring Board members are voting members. The Board may also decide in advance whether ad hoc members can vote.

Study data are accessible at all times for the team to review, and to the DSMB upon request. The PIs and co-investigators review study conduct, particularly accrual, drop-outs, and protocol deviations on a quarterly basis. The team reviews adverse events (AEs) individually in real-time and in aggregate on a quarterly basis. The PIs review serious adverse events (SAEs) in real- time. The PIs ensure that all protocol deviations, AEs and SAEs are reported to the NHLBI and IRB according to the applicable regulatory requirements (see below).

After the DSMB meets, the meeting minutes will be reviewed and approved by the DSMB Chair, then recommendations (if any) will be forwarded to the PI and to NHLBI staff. The minutes of the DSMB deliberations and recommendations (as approved by the Chair) will be submitted to the IRBs at the University of Chicago as well as to NHLBI during the annual renewals or sooner as per institutional requirements. The reports will include the following: a statement that a DSMB review of the data and outcomes across all performance sites took place on a given date; a summary of the DSMB review of the cumulative serious adverse events without specific disclosure by treatment unless safety considerations require such disclosure; and the DSMB's conclusion with respect to progress or need for potential protocol modification (e.g., continue without changes to protocol, continue with changes (specified), or stop the study).

Please see Appendix C for DMSB Charter.

10 Data Handling and Record Keeping

10.1 *Confidentiality*

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information

The rights of a research subject to revoke their authorization for use of their PHI.
This information is contained within the University of Chicago IRB Informed Consent.

10.2 *Research Material and Confidentiality*

Research material will be demographic data, wrist actigraphy recordings, sleep recordings, blood and muscle tissue samples, body weight, body composition, and indirect calorimetry data. Prescreening questions will be administered using University of Chicago REDcap, a secure portal, where the data is stored behind an encrypted firewall, and automatically backed up. All authorized study personnel at the University of Chicago will have a unique log in information to access study related data. All research material will be used only for research purposes and subject confidentiality will be protected according to guidelines. All data will be collected, stored, and managed in full compliance with HIPAA regulations as well as according to regulations by the IRB at the University of Chicago. All study personnel who will be authorized access to subject data will be trained in human subject's research (e.g., CITI certified).

10.3 *Case Report Forms*

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. Do not erase or use "white-out" for errors. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it. If the reason for the correction is not clear or needs additional explanation, neatly include the details to justify the correction.

10.4 *Records Retention*

The principal investigator will maintain records and essential documents related to the conduct of the study. These will include subject case histories and regulatory documents. Study documents will be retained for six years following the completion of the study.

11 Ethical Considerations

This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the sponsor-investigator before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the Approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or the subject's legally authorized representative, and the individual obtaining the informed consent.

12 Study Finances

12.1 *Funding Source*

The study is financed through a grant from the US National Institute of Health.

12.2 *Subject Payments*

Subjects will be paid:

- $50 for completing the Visit 1 Screening in-clinic activities
- $50 for completing the Visit 1 Screening at-home sleep study (Only subjects who qualify for the study after completing the screening in-clinic visit will be asked to complete the at home sleep study).
- $50 for completing Visit 2 CPAP Titration
- $1000 to complete The Untreated Study Period
- $1000 for completing CPAP Treated Study Period.

Subjects may be paid up to $2150 if they complete the entire study. Payments will be made after each study visit.

13 Publication Plan

The study team at the University of Chicago holds the primary responsibility for publication of the results of the study.

14 References

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15 Appendixes

A. Informed Consent Form

B. Schedule of Events (schema)

C. DSMB Charter

D. Questionnaires