Protocol Title: Randomized Controlled Trial Examining the Effects of Meal Timing among Obese Individuals

NCT Number: NCT03354169

Principal Investigators: Dr. Kelly Allison and Dr. Namni Goel

Version Date: 6.10.21

Modification

Basic Info

Confirmation Number:	**deaciahh**
Protocol Number:	**828317**
Created By:	**RUGGIERI, MADELYN**
Principal Investigator:	**ALLISON, KELLY C**
Protocol Title:	**Randomized Controlled Trial Examining the Effects of Meal Timing Among Obese Individuals**
Short Title:	**Daytime vs. Delayed Eating Among Obese Adults**
Protocol Description:	**To determine whether timing of eating affects weight, adiposity, energy metabolism, and gene expression. Obese participants will be provided isocaloric meals and snacks to be consumed in one of two prescribed eating conditions -- daytime eating and delayed eating -- in a randomized, cross-over design.**
Submission Type:	**Biomedical Research**
Application Type:	**FULL**

PennERA Protocol Status

Approved

Resubmission*
No

Are you submitting a Modification to this protocol?*

Yes

Current Status of Study

Study Status

Currently in Progress

If study is currently in progress, please enter the following

Number of subjects enrolled at Penn since the study was initiated

32

Actual enrollment at participating centers

0

If study is closed to further enrollment, please enter the following

Number of subjects in therapy or intervention

0

Number of subjects in long-term follow-up only

0

IRB Determination
If the change represents more than minimal risk to subjects, it must be reviewed and approved by the IRB at a convened meeting. For a modification to be considered more than minimal risk, the proposed change would increase the risk of discomfort or decrease benefit. The IRB must review and approve the proposed change at a convened meeting before the change can be implemented unless the change is necessary to eliminate an immediate hazard to the research participants. In the case of a change implemented to eliminate an immediate hazard to participants, the IRB will review the change to determine that it is consistent with ensuring the participant′s continued welfare. Examples: Convened Board Increase in target enrollment for investigator initiated research or potential Phase I research Expanding inclusion or removing exclusion criteria where the new population may be at increased risk Revised risk information with active participants Minor risk revisions that may affect a subject′s willingness to continue to participate Expedited Review Increase in target enrollment at Penn where overall enrollment target is not exceeded or potentially sponsored research Expanding inclusion or removing exclusion where the new population has the same expected risk as the previous, based on similarities of condition Revised risk information with subjects in long-term follow-up Minor risk revisions with no subjects enrolled to date
Expedited Review

Modification Summary
Please describe any required modification to the protocol. If you are using this form to submit an exception or report a deviation, enter 'N/A' in the box below.
We are adding Laura Ospina to the personnel section of the study. Laura will be a clinical research coordinator on the study. We are removing Sarah Badawi from the study personnel section of the study.

Risk / Benefit
Does this amendment alter the Risk/Benefit profile of the study?
No

Change in Consent
Has there been a change in the consent documents?
No

If YES, please choose from the options below regarding re-consenting

Deviations

Are you reporting a deviation to this protocol?*

No

Exceptions

Are you reporting an exception to this protocol?*

No

Protocol Details

Resubmission*
Yes

Hospital Sites
Will any research activities and/or services be conducted at a Penn Medicine affiliated hospital site?
Yes

Active Hospital Sites

Hospital of the University of Pennsylvania (HUP) ***Primary***

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Responsible Org (Department/School/Division):

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Key Study Personnel

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HS Training Completed:	**Yes**
Training Expiration Date:	**04/10/2020**
Name of course completed:	**CITI Protection of Human Subjects Research Training - ORA**

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Training Expiration Date:	**11/11/2019**
Name of course completed:	**CITI Protection of Human Subjects Research Training - ORA**

Disclosure of Significant Financial Interests*
Does any person who is responsible for the design, conduct, or reporting of this research protocol have a **FINANCIAL INTEREST**?
No

Penn Intellectual Property*
To the best of the Principal Investigator's knowledge, does this protocol involve the testing, development or evaluation of a drug, device, product, or other type of intellectual property (IP) that is owned by or assigned to the University of Pennsylvania?
No

Certification
I have reviewed the *Financial Disclosure and Presumptively Prohibited Conflicts for Faculty Participating in Clinical Trials* and the *Financial Disclosure Policy for Research and Sponsored Projects* with all persons who are responsible for the design, conduct, or reporting of this research; and all required Disclosures have been attached to this application.
Yes

Biomedical Research

Clinical Trial*
Is this a clinical trial?
Yes

If Yes, please be aware that for each clinical trial conducted or supported by a Federal department or agency, one IRB-approved informed consent form used to enroll subjects must be posted by the awardee or the Federal department or agency component conducting the trial on a publicly available

Federal Web site that will be established as a repository for such informed consent forms.

Investigator Initiated Trial*
Is this an investigator initiated trial?
No

Drugs or Devices*
Does this research study involve Drugs or Devices?
No

IND Exemption

For studies that fall under an IND exemption, please provide the number below

For studies including IND or IDE's, please provide the number(s) below

IDE Review*
NOTE: For research involving investigational devices, you are required to review the guidance on Managing Research Device Inventory. Consult the Penn Manual for Clinical Research: https:// www.med.upenn.edu/pennmanual/secure/investigational-product-management-at-sites-not-using-investigational-drug-services-(ids).html Please check the box Yes if you have reviewed the guidance.
Yes

Research Device Management*
Please indicate how research device(s) will be managed.
Not Applicable (no investigational devices)

Drug, Herbal Product or Other Chemical Element Management *
Please indicate how drugs, herbal products or other chemical entities will be managed.
Not Applicable (no drugs, herbal products or other chemical entities)

Radiation Exposure*
Are research subjects receiving any radiation exposure (e.g. X-rays, CT, Fluoroscopy, DEXA, pQCT, FDG, Tc-99m, etc.) that they would not receive if they were not enrolled in this protocol?
Yes

Gene Transfer*
Does this research involve gene transfer (including all vectors) to human subjects?
No

Human Source Material*
Does this research include collection or use of human source material (i.e., human blood, blood products, tissues or body fluids)?
Yes

CACTIS and CT Studies*
Does the research involve Center for Advanced Computed Tomography Imaging Services (CACTIS) and CT studies that research subjects would not receive if they were not part of this protocol?
No

CAMRIS and MRI Studies*
Does the research involve Center for Advanced Magnetic Resonance Imaging and Spectroscopy (CAMRIS) and MRI studies that research subjects would not receive if they were not part of this protocol?
No

Investigational Agent or Device within the Operating Room*
Does the research project involve the use of an investigational agent or device within the Operating Room?
No

Cancer Related research not being conducted by an NCI cooperative group*
Does this protocol involve cancer-related studies in any of the following categories?
No

Processing of Materials*
Will the research involve processing (such as over encapsulating, or compounding)?
No

In-House Manufacturing of Materials*
Will the research involve processing (such as over encapsulating, or compounding)?
No

Medical Information Disclosure*
Does the research proposal involve the use and disclosure of research subject's medical information for research purposes?
Yes

If the answer is YES, indicate which items is is provided with this submission:

Modified research informed consent document that incorporates HIPAA requirements

CTRC Resources*
Does the research involve CTRC resources?
Yes

Pathology and Laboratory Medicine Resources*
Will samples be collected by hospital phlebotomy and/or processed or analyzed by any of the clinical laboratories of the University of Pennsylvania Health System?
No

Research Involves Apheresis, Cell Collection, and/or Blood Product Collection*
Does this research involve collection of blood products in the Penn Donor Center and/or the use of apheresis for treatment or collection of cells or other blood components?
No

Research involving blood transfusion or drug infusions*
Will your research involve blood transfusion or infusion of study drug in 3 Ravdin Apheresis Unit for research purposes?
No

Trial in Radiation Oncology
Is this research a prospective trial being done in Radiation Oncology, and if so, has this protocol been approved by the Radiation Oncology Protocol committee?
N/A

Study in Radiation Oncology
Is this research a retrospective study being done in Radiation Oncology, and if so, has this project been reviewed by the Radiation Oncology Clinical Research Group?
N/A

Use of UPHS services*
Does your study require the use of University of Pennsylvania Health System (UPHS) services, tests or procedures*, whether considered routine care or strictly for research purposes?
Yes

Primary Focus*
Mechanistic or physiologic study in human subjects (T1 Translational research in humans or Phase I drug research)

Protocol Interventions

	Sociobehavioral (i.e. cognitive or behavioral therapy)
	Drug
	Device - therapeutic
	Device - diagnostic (assessing a device for sensitivity or specificity in disease diagnosis)
	Surgical
x	**Diagnostic test/procedure (research-related diagnostic test or procedure)**
x	**Obtaining human tissue for basic research or biospecimen bank**
x	**Survey instrument**
	None of the above

The following documents are currently attached to this item:

There are no documents attached for this item.

Sponsors

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Department budget code

400 - 400 - 4 - 572667 - xxxx - 2810 - 2947

Funding Sponsors

Name:	**NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES/NIH/DHHS**
Type:	**UPENN Federal**

Funding sponsors billing address
If you have selected a commercial or industry sponsor, please provide the appropriate address and contact information for the Sponsor for the purposes of billing for IRB review fees (initial review, continuing review and convened modification fees apply here). If the Sponsor is not industry/ commercial, this information is not necessary to provide with your application.

Funding sponsors gift
Is this research being funded by a philanthropic gift?
No

Regulatory Sponsor

Name:	**NATIONAL INSTITUTES OF HEALTH**
Type:	**UPENN Federal**

IND Sponsor

none

400 - 400 - 4 - 572667 - xxxx - 2810 - 2947

Industry Sponsor

None

Project Funding*
Is this project funded by or associated with a grant or contract?
Yes

Selected Proposals

Proposal No	Title
10061380	**Impact of Daytime vs. Delayed Eating Schedule on Weight and Metabolic Markers Among Obese Persc**

Sponsor Funding
Is this study funded by an industry sponsor?
No

Status of contract

The following documents are currently attached to this item:

There are no documents attached for this item.

Multi-Center Research

Penn as lead
1. Is this a multi-center study where Penn is serving as the Lead Site or the Penn PI is serving as the Lead Investigator?
No

Management of Information for Multi-Center Research

Penn irb of record
2. Is this a multi-center study where the Penn IRB will be asked to serve as the IRB of Record for other external study sites?
No

Other Sites

No other sites

Protocol

Abstract
The timing of food consumption is now recognized as a significant contributor to body weight regulation and metabolic functioning. However, most studies have been in rodents or normal weight persons. The proposed study extends this work, including Drs. Allison and Goel's previous work on the randomized study of daytime vs. delayed eating among healthy adults, to metabolically healthy persons

with obesity, but who remain at risk of developing Metabolic Syndrome (MetS) and diabetes. The study would represent the first randomized controlled experimental study in obese adults to provide isocaloric meals and snacks to participants in a free-living environment over a 8-wk period on each prescribed eating condition: daytime eating and delayed eating.

Objectives

Overall objectives
To determine if timing of food consumption (daytime vs. delayed eating) affects body mass, adiposity, energy homeostasis, changes in circadian rhythms, cycling of gene expression, insulin sensitivity, and free fatty acid dynamics by enrolling 40 men and women with obesity without Metabolic Syndrome (a cluster of conditions-increased blood pressure, high blood sugar, excess abdominal body fat, and abnormal cholesterol or triglyceride levels-occurring together that increase the risk of cardiovascular disease and diabetes) in a randomized cross-over design study.

Primary outcome variable(s)
Primary outcome variables include body weight (kg) to measure body mass, results from the Dual Energy X-ray Absorptiometer (DEXA) to measure adiposity, and resting energy expenditure and fuel oxidation from indirect calorimetry to measure energy homeostasis. These measures will be taken pre and post each of the eating conditions during the assessment visits to determine changes due to timing of eating.

Secondary outcome variable(s)
Secondary outcome variables include transcriptomics from blood samples collected every 4h at 0800h, 1200h, 1600h, 2000h, 2400h, 0400h and 0800h during the assessment visits, frequently-sampled intravenous glucose tolerance (FSIGT) tests, and transcriptomics from adipose tissue samples collected once each assessment visit. All of the secondary outcome variables are measured at the assessment visits, which occur pre and post eating conditions (daytime and delayed) to determine changes due to the timing of eating.

Background
A. Significance A.1. Metabolically Healthy Obesity and Obesity with the Metabolic Syndrome (MetS) Obesity affects 38% of the U.S. population (5) and is related to many serious medical comorbidities, including type 2 diabetes, heart disease, some cancers, osteoarthritis, and obstructive sleep apnea (6,7). However, approximately 10-30% of persons with obesity remain metabolically healthy (8), characterized by decreased visceral and liver fat, C-reactive protein (CRP), and mean adipocyte size, and increased serum adiponectin and adipocyte insulin sensitivity (9). The other 70-90% of obese individuals have MetS, a cluster of conditionsincreased blood pressure, high blood sugar, excess abdominal body fat, and abnormal cholesterol or triglyceride levelsoccurring together that increase the risk of cardiovascular disease and diabetes (8). Behavioral weight loss approaches are effective at helping persons reduce weight by 5-10% of their initial body weight (7), but obesity prevention remains a primary public health goal, along with identifying behavioral strategies to optimize weight loss and maintenance. Of the many factors contributing to the obesity epidemic, the timing of food consumption is recognized as a significant contributor to body weight regulation (1-4,10). A.2. Circadian Regulation of Feeding and Metabolism The underlying mechanisms governing the circadian rhythms of fundamental processes such as sleeping and eating, are well characterized. The circadian system enables organisms to synchronize behaviors, metabolism, and physiological processes to sleep-wake cycles (11,12). The core clock mechanism is based on a feedback loop involving the transcription factors BMAL1, CLOCK, and NPAS and their targets (11). Circadian rhythms are ordered hierarchically in mammals with the hypothalamic suprachiasmatic nucleus (SCN) controlling a network of central and peripheral clocks. The SCN responds primarily to light and synchronizes behavioral and physiological rhythms via circadian oscillations in extra-SCN brain circuits and peripheral tissues (11). When food is abundant and animals are kept under normal light-dark (LD) cycles, photoperiod is the primary zeitgeber (time-giver) for the master clock in the SCN, and produces a near-24 rhythm in feeding behavior and metabolism (11). Macronutrients (i.e., glucose, fatty acids, and amino acids) and nutrient-sensing molecules (e.g., AMPK, SIRT1, mTOR, and CRTC2) entrain central and peripheral clocks, changing the timing and amplitude of gene expression rhythms and metabolic pathways (11). However, a disruption in the timing of food availability induces another regulatorthe food entrainable oscillator (13-15). Unlike the SCN, peripheral clocks in the liver, other organs, and the gut microbiome respond to the timing of feeding (12). Rodent studies have demonstrated that eating out of phase

promotes obesity, insulin resistance, fat storage, and inflammation (e.g.,16-18). A.3. Influence of Delayed Eating in Humans with Shifted or Restricted Sleep Delays in the usual daytime eating pattern increase the risk of obesity and MetS in humans (11,19). Circadian misalignment resulting from shift work and sleep disorders, alters the circadian rhythms of leptin, cortisol, glucose, adiponectin, and resistin, and is related to insulin resistance, diabetes, dyslipidemia, and cardiovascular morbidity (19-26). Short sleep duration is associated with increased appetite and caloric intake, eating late at night, weight gain, and obesity (20,21,27,28). Delayed sleep timing is also related to poorer diet and later eating times, which are related to higher BMI (29). Although these findings indicate that disrupting normally-timed sleep-wake cycles impact weight, adiposity, and metabolism, it is unclear whether meal timing independently plays a causal role in metabolic dysregulation when sleep-wake cycles are held constant among persons with obesity. A.3a. Human Phenotype of Delayed Eating Night Eating Syndrome (NES). The human phenotype of a delayed pattern of eating is consistent with NES, defined by evening hyperphagia (consumption of 25% of daily caloric intake after dinner) and/or nocturnal ingestions 2/wk (30). NES is positively related to BMI (31,32) in epidemiological (33-35) and clinical (36-39) studies. While MPI Allison and colleagues have shown lower sleep efficiency in NES (40), sleep onset and offset is similar to control participants in inpatient (40) and outpatient (41) settings, even with increased caloric intake (42). Despite the lack of delayed sleep, MPIs Allison and Goel reported that persons with NES showed attenuated circadian rhythms for food intake, cortisol, ghrelin, and insulin, but increased TSH amplitude during a 24h blood draw with ad lib food access (43). Those with NES also showed phase delays of leptin, cortisol, insulin, and melatonin, with a phase inversion of glucose and a phase advance in ghrelin, likely related to their ad lib food access and limited overnight fasting period. Thus, results from various populations and methods strongly suggest that nighttime eating may contribute to weight gain or maintenance of higher weight. Gluck et al. (44) found subjects who ate between 2300h-0500h during a 3-day inpatient stay had higher 24-h respiratory quotients and carbohydrate oxidation rates, and lower fat oxidation rates than those who did not eat between 2300-0500h, suggesting a phenotype of increased energy intake and weight. Further, diabetic patients with nighttime eating are more likely to have HbA1c values 7 and 2 diabetic complications (45). Moreover, the presence of morning anorexia is related to MetS, and higher scores on the Night Eating Questionnaire are related to higher blood pressure among women, and higher waist circumference and triglycerides among men (46). Collectively, these studies demonstrate the link between night eating behaviors and increased weight and metabolic dysfunction. Therefore, it is important to understand the impact of the timing of eating on weight and energy metabolism, independent of interrupted or phase-shifted sleep or psychiatric distress (e.g., in NES). A.3b. Delayed Eating in Healthy Normal Weight Samples. Several studies have demonstrated improvements in metabolic function with a daytime compared to a delayed eating schedule using different paradigms among healthy, normal weight adults. Total daily caloric intake is negatively related to breakfast intake, but positively related to the proportion of food consumed at night (47). A study of 420 Spaniards seeking weight loss with a Mediterranean diet found that late eaters (eating the midday meal after 1500h), compared to early eaters, lost significantly less weight during the 5 mo study, despite similar self-reported energy intake, sleep duration, macronutrient content, estimated energy expenditure, and appetitive hormone profiles (48). A handful of experimental studies in humans have tested the effect of daytime vs. delayed eating on weight, some using a randomized cross-over design (49-54). Qin et al. (49) assessed 7 students assigned to typical daytime vs. delayed eating schedules for 3 wk each. After the delayed condition, melatonin and leptin peaks were attenuated, glucose increases were maintained across the early morning hours, and insulin secretion decreased, suggesting impaired insulin sensitivity. Hibi et al. (50) studied 11 females who ate either a morning (1000h) or evening (2300h) snack in addition to their 3 usual meals for 13d each. The evening, compared to the daytime snack condition decreased fat oxidation and increased total and LDL cholesterol, but glucose and insulin levels did not differ (50). LeCheminant et al. (51) examined 27 males who were prohibited from eating between 1900h-0600h for 2 wk, or ate as per their usual schedule for 2 wk, with a 1-wk intervening washout period. They consumed 2420 kcals in the restricted vs. 2664 kcals in the usual eating condition. Weight change was -0.04 kg for the restricted condition and +0.06 kg for usual eating condition. Thus, the short-term evening food restriction resulted in a small, but likely clinically relevant calorie and weight reduction. Yoshizaki et al. (52) randomized 14 males who were habitual breakfast skippers to 3 meals at either 0800h, 1300h, and 1800h, or 1300h, 1800h, and 2300h for 2 wks. Food was provided and sleep was held constant from 2400h-0600h. The early, daytime eating schedule lowered triglycerides and total and LDL cholesterol, but did not affect blood glucose, NEFAs, insulin, or HOMA-IR. Another study (53) assigned females to an early (1300h) vs. late (1600h) lunch for 1 wk each, keeping breakfast and dinner times fixed and providing food for these meals. The late-lunch condition was associated with decreased resting energy expenditure, fasting carbohydrate oxidation, glucose tolerance, and thermal effect of food, and a blunted daily cortisol

profile. In a final study, 20 participants received an identical meal either at 0800h or at 2000h, 1 wk apart. Resting metabolic rate was increased after the morning compared to the evening meal, and glucose and insulin responses were more controlled after the morning meal (54). These studies demonstrate that delayed eating increases weight and produces metabolic dysfunction, while daytime eating improves these parameters. However, in contrast to the current proposal, only 2 of these studies controlled for, or carefully monitored, calories, macronutrient content, activity levels, or timing of sleep-wake cycles, all were of short duration (3 wk maximum), most used small sample sizes, and none used FSIGT or examined gene expression. It is critical to study weight and metabolic changes and their underlying circadian mechanisms, including insulin sensitivity and free fatty acid dynamics and gene expression in AT and blood, under conditions controlling all of these factors, while also testing an eating schedule over a longer time period. A.3c. Timed Eating in Obese and Overweight Persons with MetS and Bariatric Patients. Jakubowicz et al. (55) tested the timing of calorie distribution during a 12-wk weight loss intervention, providing the largest meal of the day (700 kcals out of 1400 daily kcals) either at breakfast or dinner among participants with BMI 25kg/m2 and MetS. The breakfast group showed greater weight loss and improvements in fasting glucose, insulin, ghrelin, and triglycerides than the dinner group. An oral glucose tolerance test also showed greater decreases in glucose and insulin for the breakfast group. Improvements were also found in postprandial hyperglycemia in a similar study of 1-wk duration among persons with type 2 diabetes (56). Ruiz-Lozano et al. (57) also examined the impact of meal timing on Spanish bariatric patients, finding that those persons who were considered poor weight loss responders to surgery were more likely to consume their main meal after 1500h, than persons who ate their main meal before 1500h. These studies focused on the timing of the main meal of the day, with compelling effects; they suggest that loading calories to the beginning of the day is beneficial for weight and metabolism, including glucose regulation. We propose building upon these studies using metabolically healthy adults with obesity to identify the impact of shifting eating hours, not the amount of energy consumed, and examining the circadiandependent mechanisms underlying changes to weight and metabolism in this population. A3d. Time-Restricted Feeding and Intermittent Fasting. Time-restricted feedinglimiting eating to a certain number of hours per day (e.g., 0800h-1400h) is an increasingly popular approach for weight management. It also refers to intermittent fasting, where persons fast (500-700 kcals) for a set number of days/wk, and then eat ad lib the remaining days (e.g., 2d of fasting and 5d of ad lib eating). These approaches were derived from rodent models (58) and require more research for definitive conclusions on their benefits in humans (1), particularly among obese persons. Notably, our R21 and the current proposed study hold the total number of hours of eating constant at 11h/d (0800h-1900h or 1200h-2300h), while keeping the sleep-wake cycle constant (2300h-0700h). A.4. Transcriptomics (Gene Expression) Transcriptomics or expression profiling is the study of the complete set of RNA transcripts produced by the genome (transcriptome), under specific circumstances or in a specific cell, using high-throughput methods such as microarray analysis or short-read high-throughput sequencing (RNASeq). Comparison of transcriptomes allows for the identification of genes that are differentially expressed in distinct cell populations or in response to different conditions (59). A.4a. Adipose Tissue (AT) Transcriptome: Links to Obesity, Circadian Rhythms and Timed/Delayed Eating in Humans. AT and Obesity. Obese persons have an excess amount of AT fat, a major site of energy storage that has a role in the regulation of metabolism through the release of adipokines. AT dysregulation contributes to the development of obesity-related co-morbidities (60-62). AT constitutes an important source of circulating RNAs, which can regulate gene expression. Such AT gene expression is differentially altered during various dietary weight loss interventions in obese persons (63-69) and in short- and long-term overfeeding (70,71). AT gene expression has been used to distinguish metabolically healthy obese persons (72) and has been associated with insulin sensitivity, and plasma lipid and glucose levels in this population (64,73). AT and Circadian Rhythms. AT is a peripheral oscillator capable of modulating central core clock genes (74). Clock genes control some of these adipokines, and their expression in AT exhibits diurnal variation in obese persons (75-77). In addition, ex vivo explants demonstrate the presence of peripheral circadian oscillators in human AT that can function independently of the central (SCN) circadian mechanism (78), with adiponectin-related (79) and glucocorticoid-related gene expression (80) showing circadian rhythmicity. In addition, AT from obese persons shows a robust insulin signaling circadian rhythm (81). Timed/delayed eating and AT Gene Expression. As reviewed above (sections A.3b. and A.3c.), timed/delayed eating impact circadian rhythms. Given these effects, and the fact that AT shows circadian rhythms in gene expression, timing of eating should affect AT gene expression. Only one study in mice has examined this question. Hatori et al. (18) investigated mice who received ad lib or time-restricted feeding (TrF) of a high-fat diet for 8h/d and consumed equivalent calories; those on the TrF showed improvements in AT gene expression. How delayed timed eating affects AT gene expression in humans remains unknown. As such, we will examine the circadian mechanistic role of AT gene expression underlying the weight and

metabolic changes associated with the daytime and delayed eating conditions. We hypothesize clock genes and adipocyte genes will change in the daytime vs. delayed conditions, allowing discrimination of the 2 states. These altered genes may serve as therapeutic, pharmacological targets (82) and biomarkers for obesity-related complications. We will also correlate AT gene expression with fasting lipid and glucose levels. In addition, as AT and blood will be collected on the same day, we will compare pre-post changes in AT and blood expression using meta-analyses, to identify how different tissues relate to each other as a function of eating condition, and to identify tissue-specific changes resulting from a daytime vs. delayed eating schedule. Such knowledge would also be advantageous for collection methods, given the limited accessibility and subject burden involved in AT biopsies vs. the minimally invasive, accessible collection of blood. Finally, genes that emerge across AT and blood would be viable biomarkers and targets for future studies. A.4b. Blood Transcriptome: Links to Obesity, Circadian Rhythms and Timed/Delayed Eating in Humans. Blood Transcriptome and Obesity. Blood gene expression profiling in obese persons has identified distinct biological pathways associated with obesity (83-85) and BMI (86). In addition, dietary patterns, dietary challenges and high fat diets all alter blood gene expression (87-89). Blood Transcriptome and Circadian Rhythms. Transcriptomic studies using peripheral blood have shown that gene expression and rhythmicity are affected by desynchrony between sleep and circadian phase (i.e., mistimed sleep), constant routines, and sleep loss, whereby the number and/or amplitude of genes exhibiting circadian rhythmicity is altered (59,90-98). These studies highlight the gene expression interrelationships between circadian rhythmicity and sleep homeostasis in blood. We propose to extend the analysis of blood transcriptome in circadian rhythm studies to obesity and timed eating paradigms. Timed/Delayed Eating and Blood Gene Expression. Since timed/delayed eating impacts circadian rhythms (sections A.3b. and A.3c.), and blood shows circadian rhythms in gene expression, the timing of eating should affect blood gene expression. Notably, how delayed timed eating affects blood gene expression in humans remains unknown. As such, we will compare the daytime vs. delayed eating schedule on the phase and cycling amplitude of gene expression profiles from blood. We hypothesize central and peripheral clock genes will show significant changes in the daytime vs. delayed conditions, allowing discrimination of the 2 states. In addition, we will cluster genes by phase for each 4h sample bin and compare these clusters with circadian rhythm hormone phase profiles using correlational analysis to identify how different blood markers relate to each other as a function of eating condition; this may allow for identification of additional genes implicated in timed eating. A5. Study Team Experience. The proposed research will be conducted by investigators from UPenns Center for Weight and Eating Disorders (CWED), Division of Sleep and Chronobiology, Division of Endocrinology, Diabetes, and Metabolism, and Department of Neuroscience and CHOPs Department of Biomedical and Health Informaticsall within close proximity (10 min walk). MPIs Allison and Goel have collaborated on prior research efforts and have established a strong working relationship. They have a R21 grant together and have published on the circadian and metabolic characteristics of NES (43) and a review on delayed eating (2). MPI Goel has collaborated with Co-I Sehgal on several projects including recent investigations on the human and rat microbiome (99) and metabolome (100). MPI Goel has also collaborated with Co-I Taylor on a recent investigation of changes in human and rat microRNAs during sleep loss (101). MPI Allison has worked with Co-I Rickels on her current R21, as he served as the physician contact for the inpatient assessments; Co-I Rickels has also worked with our group at CWED on a study using FSIGT in bariatric and behavioral weight loss patients (102). The investigators have extensive research experience in eating behaviors, weight management, and obesity in humans (Allison); circadian rhythms, sleep-wake and energy balance in humans (Goel); molecular and cellular mechanisms of circadian rhythms (Sehgal); endocrinology and metabolism in humans (Rickels); and biomedical and health bioinformatics (Taylor). Our R21 study; human studies of NES and metabolic changes with sleep loss, weight loss, and metabolic disorders (30,40,41,103-108); long-term, experimental measurement of human circadian rhythms, sleep, metabolism and activity (109-120); studies on insulin sensitivity, free fatty acid dynamics and AT (121-123); and studies on transcriptomics (124-126), bring a unique set of skills to examine this translational topicthe impact of the timing of eating on body mass and fat, energy metabolism and hormonal regulation, and its circadian mechanisms in persons with obesity. A.6. Scientific and Public Health Relevance of the Proposed Project. The timing of eating impacts weight and metabolic functioning, but most studies have been in rodents or normal weight persons. The proposed study extends this work to metabolically healthy persons with obesity, but who remain at risk of developing MetS and diabetes. This translational human experiment controlling for eating and sleep timing, caloric intake, and exercise will uncover numerous potential circadian mechanisms resulting from the metabolic- and weight-related impact of a daytime vs. delayed eating schedule that contribute to the pathophysiology of obesity. Determining such circadian-dependent mechanisms using deep phenotyping will identify targets for medical interventions for obesity and for prevention of the MetS

and diabetes, and will also inform clinical treatment guidelines regarding behavioral strategies that could be recommended by public health platforms and by medical personnel to modify the timing of eating for management of weight and metabolic disease.

Study Design

Phase*
Not applicable

Design
The study is a randomized within-subjects cross-over design.

Study duration
The total duration of subject participation will be approximately 19 wks: baseline assessments will take approximately 1 wk, the first eating condition will last 8 wk, followed by a 2-wk washout, and then followed by the second 8-wk eating condition (see Table 2 for study events for participants). The timeline of the proposed study will be 5 years to enroll and complete all subjects.

Resources necessary for human research protection
Describe research staff and justify that the staff are adequate in number and qualifications to conduct the research. Describe how you will ensure that all staff assisting with the research are adequately informed about the protocol and their research related duties. Please allow adequate time for the researchers to conduct and complete the research. Please confirm that there are adequate facilities for the research.
The proposed research will be conducted by investigators from UPenns Center for Weight and Eating Disorders (CWED), Division of Sleep and Chronobiology, Division of Endocrinology, Diabetes, and Metabolism, and Department of Neuroscience and CHOPs Department of Biomedical and Health Informaticsall within close proximity (10 min walk). MPIs Allison and Goel have collaborated on prior research efforts and have established a strong working relationship. They have a R21 grant together and have published on the circadian and metabolic characteristics of NES (43) and a review on delayed eating (2). MPI Goel has collaborated with Co-I Taylor (at CHOP)on a recent investigation of changes in human and rat microRNAs during sleep loss (101). MPI Allison has worked with Co-I Rickels on her current R21, as he served as the physician contact for the inpatient assessments; Co-I Rickels has also worked with our group at CWED on a study using FSIGT in bariatric and behavioral weight loss patients (102). The investigators have extensive research experience in eating behaviors, weight management, and obesity in humans (Allison); circadian rhythms, sleep-wake and energy balance in humans (Goel); endocrinology and metabolism in humans (Rickels); and biomedical and health bioinformatics (Taylor). Our R21 study; human studies of NES and metabolic changes with sleep loss, weight loss, and metabolic disorders (30,40,41,103-108); long-term, experimental measurement of human circadian rhythms, sleep, metabolism and activity (109-120); studies on insulin sensitivity, free fatty acid dynamics and AT (121-123); and studies on transcriptomics (124-126), bring a unique set of skills to examine this translational topicthe impact of the timing of eating on body mass and fat, energy metabolism and hormonal regulation, and its circadian mechanisms in persons with obesity. In addition, all study staff will receive a copy of the protocol which will also be reviewed with them in detail to ensure they are adequately informed about the protocol and their individual duties.

Characteristics of the Study Population

Target population
Obese men and women ages 21-50 years old with a BMI between 30-50kg/m2 without metabolic syndrome.

Subjects enrolled by Penn Researchers
40

Subjects enrolled by Collaborating Researchers
0

Accrual

Participants will be recruited through postings on campus and nearby workplaces, pamphlets to Penn Medicine practices, internet advertisements, and local media appearances. Participants will live/work within a 5 mi radius of the Hospital of the University of Pennsylvania for compliance with study visits and for ease of food delivery. UPenn currently employs 17,500 faculty and staff. Penn Medicine employs 21,626 people, and West Philadelphia (zip code 19104) has an adult population of about 54,718 (36% white, 40% black, 15% Asian; 50% female) (131). They will be required to have access to a personal electronic device for taking and sending photographs to study staff and receiving and responding to a link to REDCap (Research Electronic Data Capture) with daily queries regarding eating, sleep, and exercise parameters. We required this for our R21, and did not encounter issues; we expect this will not greatly impact recruitment. Power analysis for the outcome variables are based on our R21 data, which was designed to generate effect sizes (Cohens d; 127) for a R01 application; it uses the same study design sequence consisting of 2 orders (AB/BA design). Table 1 (see section C.1.) summarizes the power estimates, all assuming a 90% retention rate (n=36), a 2-sided paired t-test, and =0.05 (G*Power 3.1 program; 159). Power is above 80% for 7 of the 11 variables proposed, including weight, RQ, total cholesterol, triglycerides, insulin, trunk fat%/leg fat%, and circadian phase of leptin. Gene expression studies have not been done previously using our proposed design. However, within-subjects blood transcriptome studies in the circadian rhythm and obesity fields have detected gene expression changes using sample sizes of n=9-28 (84,90-92,94,95,98); similarly, within-subjects AT transcriptome studies in obese subjects have detected gene expression changes using sample sizes of n=6-20 (64,65,67,68). Thus, we anticipate having enough power to detect AT and blood expression changes (Specific Aims 2 and 3) between the daytime and delayed conditions using a within-subjects design and a sample size of n=36. Similarly, insulin sensitivity and free fatty acid dynamics studies have not been done previously using our proposed design. However, FSIGT studies have detected significant insulin sensitivity and free fatty acid dynamics changes using sample sizes of n=10-20 (121-123); thus, we anticipate having enough power to detect these changes (Specific Aim 3) between the daytime and delayed conditions using a within-subjects design and a sample size of n=36. Therefore, we will have sufficient power to detect significant differences between conditions in almost all of outcome variables for Specific Aims 1, 2 and 3, assuming a 10% study dropout rate (final sample size, n=36).

Key inclusion criteria

We will recruit adults of all races and ethnicities, ages 21-50y, BMI of 30-50 kg/m2, and stable weight (±10 lb) over the previous 6 mo.

Key exclusion criteria

Exclusion criteria include unstable, serious medical conditions; use of medicine linked to weight gain/ loss; cardiac conditions; cancer, with the exception of a remission period of or equal to 5 years or skin cancer without adjuvant treatment, diabetes, or autoimmune disease; use of illicit drugs, melatonin, diuretics or hypnotics; current weight loss program; presence of an untreated sleep disorder (determined by surveys and actigraphy); shift work; extreme chronotypes; habitual waking outside of 0600h-0930h; habitual bedtime 2200h or 2400h; and sleep duration of 6.5 or 8.5 h/night. Psychiatric exclusions will be: depression (Patient Health Questionnaire-9 (133) score 9), lifetime bipolar disorder, psychosis, or eating disorder; or any other severe psychiatric disorder judged to interfere with study adherence as assessed by the MINI International Neuropsychiatric Interview (134). Exclusions also include structured exercise 3 d/wk, for 30 min measured by exercise logs and actigraphy; normal activity levels are required during the study (±30 min/wk of baseline level). Participants will be excluded if they are pregnant. A urine pregnancy test will be given to all women, with the exception of post-menopausal women and women who have had surgeries or treatments, such as a hysterectomy, that make them infertile, during the screening visit and each of the assessment visits. If a woman becomes pregnant while on the study, she will be asked to notify the study team and asked to consult an obstetrician or maternal-fetal specialist. She will be withdrawn from the study. The study physician or nurse practitioner will remain in contact with them to learn the outcome of their pregnancy. A urine drug test will also be collected at the screening and assessment visits to assess for illicit drug use.

Vulnerable Populations

	Children Form
	Pregnant women (if the study procedures may affect the condition of the pregnant woman or fetus) Form
	Fetuses and/or Neonates Form
	Prisoners Form
	Other
x	**None of the above populations are included in the research study**

The following documents are currently attached to this item:

There are no documents attached for this item.

Populations vulnerable to undue influence or coercion
We will exclude any participants who work in the Center for Weight and Eating Disorders, the Unit for Experimental Psychiatry, and Dr. Rickel's lab. We will not exclude other employees or students of the University of Pennsylvania. If employees or students from the University of Pennsylvania are enrolled, they will be informed that their involvement in the study in no way affects their standing with the University of Pennsylvania.

Subject recruitment
Participants will be recruited through postings on campus and nearby workplaces, pamphlets to Penn Medicine practices, internet advertisements, and local media appearances.

Will the recruitment plan propose to use any Penn media services (communications, marketing, etc.) for outreach via social media avenues (examples include: Facebook, Twitter, blogging, texting, etc.) or does the study team plan to directly use social media to recruit for the research?
Yes

Please identify which method(s) of social media you will utilize, the content of the text to be used, and the method(s) for posting this information (i.e., using Penn supported communication services). When proposing the text to utilize, please be aware of any social media limitations (i.e., number of characters allowed in a tweet) and any appropriate confidentiality practices necessary to be compliant with posting research recruitment text.*
We will advertise for the study using a Facebook advertisement using one of the advertisements submitted for approval.

The following documents are currently attached to this item:

There are no documents attached for this item.

Subject compensation*
Will subjects be financially compensated for their participation?
Yes

The following documents are currently attached to this item:

There are no documents attached for this item.

If there is subject compensation, provide the schedule for compensation per study visit or session and total amount for entire participation, either as text or separate document

Participants will be provided with all meals and snacks for a total of 16 weeks at an estimated value of $6500 ($58/day). Subjects will be compensated $500 at each assessment visit (n=4), with a $300 bonus at study end for a total of $2300.

Study Procedures

Suicidal Ideation and Behavior
Does this research qualify as a clinical investigation that will utilize a test article (ie- drug or biological) which may carry a potential for central nervous system (CNS) effect(s)?
No

Procedures
Participants will be recruited through postings on campus and nearby workplaces, pamphlets to Penn Medicine practices, internet advertisements, and local media appearances. Participants will complete an initial phone screen to determine eligibility and assess availability. Participant responses will be reviewed by the principle investigator and if they appear to be eligible, a screening visit will be scheduled. Study staff will complete a clinical interview assessing typical eating and sleep patterns, weight history, and psychiatric status. Participants will also be asked to complete several self-report questionnaires to assess mood, exercise, sleeping and eating patterns, and to examine psychological constructs related to eating and food. Participants will undergo a medical history and physical, and their weight, height, and waist circumference will be measured. Blood pressure, pulse, fasting blood work (which includes tests for a metabolic panel, fasting glucose, HbA1c, lipid panel, and CBC test), and an EKG will also be performed to exclude diabetes, MetS, and other metabolic or cardiac issues. Participants will receive logs to record food, physical activity, and sleep-wake cycles, as used in our previous studies of NES (135,136) and our R21 study, and an actigraph to wear for 10d. Upon completion, study staff will review their logs and actigraphs, and if they meet inclusion criteria, they will be scheduled to undergo the inpatient baseline assessment. After baseline assessment, participants will be randomized to begin the study with either the daytime or delayed eating condition, and they will receive their first 3d of food. The participants will also receive food, sleep, and exercise logs and actigraphs, which they will use for the study duration. Assessment Overview. Assessments will be conducted at 4 phases: 1) pre-eating condition 1; 2) post-eating condition 1; 3) pre-eating condition 2; and 4) post-eating condition 2 (Table 2). Each assessment will include 28h at the Center for Human Phenomic Science (CHPS) where participants will eat according to a standardized schedule. Participants will observe an overnight fast and arrive at CHPS at 0700h. Weight, height, waist circumference, and blood pressure will be measured. Participants will be asked to complete a series of questionnaires to assess psychological measures related to eating and food. Blood will be drawn at 4h intervals for 24h (0800h, 1200h, 1600h, 2000h, 2400h, 0400h and 0800h) to measure circadian rhythms of leptin, ghrelin, melatonin, cortisol and glucose, and of gene expression (43,90,92,95,98,137). A one-time blood sample will also be drawn for genomic analysis.. Fasting levels of glucose, insulin, adiponectin, cholesterol, triglycerides, non-esterified fatty acids (NEFAs) and CRP will be measured at 0800h. At the same 4h intervals (0800h, 1200h, 1600h, 2000h, 2400h, 0400h and 0800h), a saliva sample will be obtained using salivette salivary swabs to collect information on changes to the salivary microbiome. This involves participants placing a cotton swab called a Salivette in their mouth to absorb saliva and placing it back into a small tube to be stored; they will be asked to refrain from using toothpaste, mouthwash, or gum beginning at 11 pm the night before admission for this test. After the initial fasting blood draw at 0800h, indirect calorimetry will assess REE and fuel oxidation (RQ) at 0900h. A standardized breakfast will then be served. Next, body composition will be measured using Dual Energy X-ray Absorptiometer (DEXA). At 1200h, blood will be drawn followed by lunch and harvesting of AT at 1300h. Following the AT procedure, participants will complete three computer-based cognitive tests administered by research staff. An afternoon snack, dinner, and evening snack will be served; the last snack will be consumed by 2100h. Participants will then fast overnight in preparation for the FSIGT, and blood draws will continue every 4h. At 0800h, the FSIGT will occur, followed by breakfast, and then discharge from the CHPS. Michael Rickels, MD, will be contacted in case of medical emergency. In his absence, we will call the hospital operator at 215-662-2222 and ask to speak to the endocrinology fellow on call. Dietary Plan and Food Distribution. Research staff will deliver meals and snacks from the CHPS metabolic kitchen 2x/wk, pick up uneaten food, and collect and provide new actigraphs. Participants may also choose to pick up food themselves from the metabolic kitchen if they prefer. Menus will accompany the meals, and participants will check off each food item they consume and note any modifications to their meals and snacks. The CHPS staff will provide portion size training at baseline. Participants will purchase their own beverages and record them on their menus. Mean beverage intake, as reported at baseline, will be factored into each participants total

daily caloric and macronutrient goals. CHPS metabolic kitchen staff will provide a diet consisting of approximately 55% carbohydrate, 15% protein, and 30% fat with a eucaloric energy level. These parameters will remain constant across conditions. Study staff will send a reminder link to participants to complete queries in the REDCap system to monitor adherence to the eating, sleep, and exercise parameters. This system and the queries are identical to the ones we utilized in our R21 study and will be as follows: 1. What time did you wake up this morning?; 2. What time did you go to bed last night?; 3a. Did you eat before 8 am (for daytime condition)/12 pm (for delayed condition) or after 7 pm (for daytime condition)/11 pm (for delayed condition) yesterday? 3b. If yes, what times did you eat?; 4. How many of your 3 meals and 2 snacks did you eat yesterday?; 5. How many minutes did you exercise yesterday?. To facilitate compliance, staff will intervene the same day if participants report consuming 80% (4/5) of the provided meals and snacks, eating or sleeping outside of the assigned time windows, or exercising more than prescribed. Staff will also download actigraphy data and collect the logs weekly to monitor compliance. We successfully utilized these procedures in our R21 study and do not expect any issues to arise with an obese population. If participants consume a meal or snack outside of the provided food, they will send a picture of it using an electronic device, including a reference object, i.e., a card of known dimensions provided by the investigators to orient the staff to the portion size. This approach worked successfully in our R21 study. All uneaten food will be saved and returned to the metabolic kitchen when staff delivers new food. If it is not possible to save leftover food, participants will take a picture of the remaining food including the reference object. Kitchen staff will pre-weigh all provided items and post-weigh any non-eaten items and compute macronutrient and caloric counts for daily intake using the Nutrition Data System for Research (NDSR). [Note: participants will be provided a master meal and snack menu consisting of fresh and prepared options from which they can pick foods they find acceptable]. If participants do not like certain foods, they can alter their choices. Regular contact with participants through scheduled food deliveries and daily queries will facilitate these requests. This approach worked successfully in our R21 study. We plan to offset the high study burden by enrolling participants in close proximity to the CHPS, providing them with all meals and snacks for a total of 16 wk at an estimated value of $6500 ($58/d), and compensating them $500 at each assessment (n=4), with a $300 bonus at study end ($2300 total). After the first condition (8 wk), participants will complete assessment 2. [Rationale: We chose 8 wk for the eating conditions based on our R21 findings as well as the literature. For example, Garaulet et al. (48) did not find a separation between early and late eaters in weight loss outcomes until wk 5. Hibi et al. (50) did not observe significant differences in weight between daytime vs. nighttime snack conditions after 13d, although the daytime snack group had a slight loss and the nighttime snack group had a slight gain in weight. We are not proposing a weight loss intervention, as in Garaulet et al. (48) or Jakubowicz et al. (55), so extending the test period beyond 5 wk is merited. We believe 8 wk represents a sufficient period for weight change, and our R21 results support this claim.] A 2-wk washout will follow the first condition to ensure complete stabilization of circadian rhythms (which typically shift 1 h/d) before participants undergo assessment 3 (Table 2) and begin the second eating condition. During the 2-wk washout participants will be asked to follow their own desired eating times and eat their own food (no study food will be provided). Two weeks also will be sufficient to give participants an opportunity to eat normally before returning to the study food and schedule. In our R21 study, the circadian phase of hormones, weight and other measures did not differ between the pre-daytime and pre-delay conditions, indicating they returned to pre-condition levels. Participants will then complete the second eating condition, followed by assessment 4. Mid-condition check-in. At week 4 of each condition, participants will be asked to meet at the CHPS unit for a blind measurement of weight, to return their current actiwatch and receive a new actiwatch from the research staff, and to receive a series of questionnaires to complete over the upcoming week. This visit can be completed during one of the participant's food pick-up days, if they are opting for food pick-ups, to save the burden of an extra visit and make it most convenient for the participant. The research staff will collect the questionnaires at the following weeks food delivery/ pick-up. Adherence. Adherence to the protocol is critical for study success. We have experience working with the CHPS metabolic kitchen staff in previous studies (e.g.,103), and in our R21 study, and have found that frequent contact of participants with staff allows for regular feedback about the acceptability of the food and problem-solving regarding adhering to the eating and sleep-wake schedule. Participants will be asked to respond to 5 queries daily regarding compliance (see section C.3b.), with same-day intervention if participants report noncompliance. In addition, MPI Allison is a clinical psychologist who provides treatment for disordered eating and weight management and can help strategize with participants to adapt to each eating schedule. This regular contact, in person and electronically, will allow optimized protocol adherence. The $300 bonus will also promote retention and compliance. We expect similar adherence from the proposed sample of persons with obesity. Screening C.4a. Interviews and Surveys. Several validated measures will assess the inclusion and

exclusion criteria at the screening visit. We will interview participants using a 24-h food and sleep recall and an abridged Night Eating Syndrome History and Inventory (NESHI; 138) to assess typical eating patterns. Participants will complete the Patient Health Questionnaire (PHQ-9; 133), a 9-item well-validated questionnaire that assesses degree of depressed mood. Persons with scores 9 (minor depressive symptoms) will be excluded. The MINI (134) will assess psychiatric diagnoses. The Morningness-Eveningness Questionnaire (MEQ; 139) will be used to screen out extreme morning and evening chronotypes who would find it difficult to adhere to the sleep or eating schedules. The Pittsburgh Sleep Quality Index (PSQI; 140) measures several sleep domains; the global score for overall sleep quality distinguishes good (5) and poor sleepers (5). The Epworth Sleepiness Scale (ESS; 141) assesses daytime sleepiness; a score 10 is suggestive of sleep apnea, narcolepsy, and idiopathic hypersomnia. The Multivariable Apnea Risk Index (MAP Index; 142) is used to screen out subjects with sleep apnea risk (scores .5). We will use the MAP, PSQI, and ESS to exclude individuals with sleep disorders. If a sleep partner is available, we will also ask him/her to complete a MAP for the participant. We have successfully utilized these screening procedures in our R21 study. In addition to these questionnaires given, participants will also complete 3 other questionnaires at their screening visit to assess food related attitudes. The Behavioral Inhibition System/Behavioral Activation System (BIS/ BAS) Scale is a 24-item scale that assesses the sensitivity of systems that react to punishment (BIS) and reward (BAS) cues to better assess an underlying reward sensitivity dimension. The Sensitivity to Punishment (SP)/Sensitivity to Reward (SR) Questionnaire (SPSRQ) is a yes-no response item questionnaire that assesses individual differences in anxiety or sensitivity to the punishment dimension and impulsivity or sensitivity to reward dimension. Lastly, the Barratt Impulsiveness Scale (BIS) assesses common impulsive behaviors and preferences and impulsive personality traits. Once per assessment visit the following self-report questionnaires will be administered: 1. Food Craving Inventory - a self-report measure of general and specific food cravings; 2. Modified Yale Food Addiction Scale (mYFAS), is a 13-item scale identifying those who are most likely to be exhibiting markers of substance dependence with the consumption of high fat/high sugar foods and impairment or distress from eating 3. Temporal Experience of Pleasure Scale (TEPS) - an 18-item self-report measure assessing anticipatory and consummatory facets of pleasure 4. Distress Tolerance Scale (DTS) - measures an individuals (1) ability to tolerate emotions (tolerance); (2) assessment of the emotional situation as acceptable (appraisal); (3) level of attention absorbed by the negative emotion and relevant interference with functioning (absorption); and (4) ability to regulate emotion (regulation) 5. The Eating Inventory (EI) - measures cognitive restraint of eating, disinhibition, and hunger 6. The Power of Food Scale (PFS) - measure of individual differences in appetite-related thoughts, feelings and motivations in environments where plentiful palatable foods are constantly available. 7. The Weight Efficacy Lifestyle Questionnaire (WEL) - a 20-item questionnaire assesses the ability to resist eating in response to certain environmental situations or emotional states. It evaluates self-efficacy in five contexts: negative emotions, food availability, social pressure, physical discomfort, and positive activities. 8. Quality of Life, Enjoyment, and Satisfaction Questionnaire (QLES-Q) - measures domains associated with quality of life factors. It contains 14 items scored on a 0-5 scale with a possible range of 0-70. Lastly, before and after each meal and snack during the assessment visits, participants will be asked to complete a Visual Analogue Scale (VAS) questionnaire to assess hunger, cravings, and fullness. History and Physical. Participants will undergo a history and physical at the outpatient CHPS facility with a nurse practitioner. They will have initial labs drawn, including a metabolic panel (including fasting glucose), lipid panel, CBC, and HbA1c. The nurse practitioner will also complete an EKG (using a GE Mac 5500 HD EKG Machine) and assess blood pressure and pulse (using a Datascope Accutorr V Vital Signs Monitor) in duplicate at 1-min intervals, after a 5-min seated rest. Weight will be measured in light clothing without shoes on a digital scale (Scale-Tronix), and height will be measured on a wall-mounted stadiometer (ProDoc/Deteco). Waist circumference will be measured with a flexible tension-controlled measuring tape held at the midpoint between the iliac crest and lowest rib to the nearest 0.1 cm. MetS will be defined by the presence of 3 of the following: abdominal obesity (35 waist circumference for women/ 40 for men); elevated triglycerides (150 mg/dL and/or use of medication for dyslipidemia); low HDL cholesterol (50 mg/dL for women/40 mg/dL for men); elevated blood pressure (130/85 mm Hg and/or hypertensive medications); and elevated fasting blood glucose (100 mg/dL and/or medications to treat pre-diabetic blood glucose levels)(143). A pregnancy test will be given to women at this screening visit, with the exception of post-menopausal women and women who have had surgeries or treatments, such as a hysterectomy, that make them infertile. A urine drug test will be given as well. Outpatient Assessments Food, Sleep, and Activity Logs. Participants will complete a log at baseline, an approach we have used in our R21 and previous studies (41,103,135,136), to track their typical eating, sleep, and physical activity patterns. The food log will be analyzed for caloric and macronutrient content using the NDSR by a CHPS research dietician. Self-reported sleep and physical activity will be compared to

actigraphy data to assess sleep-wake onset and offset and minutes of deliberate physical activity. After baseline, the food log will be a menu based on the foods provided to participants along with the sleep and activity questions. Actigraphy. Wrist actigraphs with light sensors (Spectrum Pro, Philips Respironics Healthcare) will be worn continuously on the non-dominant wrist for the study duration (except when bathing). Actigraphy provides an objective, reliable and valid method for assessing circadian activity patterns, sleep-wake cycles, and light levels in free-living populations with minimal restriction on normal routines (115). Sleep onset, offset, duration, number of nighttime awakenings, and exercise bouts will be determined by actigraphy and confirmed by logs. Inpatient Assessments At each assessment, we will measure weight (kg), height (cm), blood pressure, and waist circumference (see section C.4b.). Participants will also be given a urine drug test and pregnancy test (with the exception of post-menopausal women and women who have had surgeries or treatments, such as a hysterectomy, that make them infertile). Intravenous Placement & Blood Draws for Hormones. Participants will arrive at 0700h at the CHPS and be housed in a 20 lux room (to avoid suppression of melatonin secretion). They will be placed in a supine position and an indwelling intravenous catheter will be inserted at 0730h. Blood draws of 10 ml for hormones will occur every 4h at 0800h, 1200h, 1600h, 2000h, 2400h, 0400h and 0800h. This sampling interval schedule strikes a balance between allowing for detection of circadian phase and amplitude of leptin, ghrelin, melatonin, cortisol and glucose, relative to food intake and sleep, as we and others have documented with circadian cosinor analyses (43,137). Cholesterol, triglycerides, glucose, insulin, adiponectin, NEFAs and CRP will also be measured from the initial fasting blood draw at 0800h on the first morning. No eating will be allowed after 2300h the night before and the night of the assessment. For hormones, blood will be collected in pre-cooled vacutainer tubes containing EDTA and kept on ice until centrifugation at 4oC and then stored at -80oC. Serum will be pipetted into tubes before freezing. Assays will be performed in duplicate by the Penn Diabetes Research Center Radioimmunoassay & Biomarkers Core. Blood Draws for Transcriptomics. 2.5ml whole blood samples also will be collected every 4h at 0800h, 1200h, 1600h, 2000h, 2400h, 0400h and 0800h, into PAXgene Blood RNA tubes (PreAnalytix). This sampling interval schedule allows for detection of the amplitude and circadian phase of gene expression, as has been documented with circadian cosinor analyses (90,98). The PAXgene tubes contain a reagent that helps lyse blood cells to release total RNA and stabilizes the in vivo gene transcription profile by preventing in vitro RNA degradation in blood. The blood will be mixed with this reagent by gently inverting the tube 8-10 times and then will be kept upright at room temperature (18°C to 25°C) for a minimum of 2h and a maximum of 72h. Next, the tubes will be transferred to a -20°C freezer for 24h, and then to an -80°C freezer for storage. An additional 10mL of blood will be collected during the first assessment visit and saved and stored as whole blood. This sample will later be analyzed for the entire genome to provide the researchers with more complete information as to how all genes may contribute to metabolism, sleep and circadian (24-hour) rhythms, an important area of interest to our research. Energy Expenditure. After completion of the first blood draw, participants will remain in bed in a fasted state. At 0900h, resting metabolic rate will be assessed by indirect calorimetry (Parvo Medics TrueOne 2400, Sandy, UT). The Parvo Medics metabolic monitor is an integrated metabolic measurement system which contains O2 and CO2 analyzers, a device for measuring ventilation rates, a gas sampling system, and a computer interface that processes raw data. Resting energy expenditure will be assessed continuously by measuring the volume and the O2 and CO2 concentrations of expired air samples, and will be calculated based on the average VO2 and RQ measures which can be converted to energetic equivalents (kcal/day) (144). Dual Energy X-ray Absorptiometer (DEXA). Following the metabolic testing and breakfast, DEXA (Hologic Discovery Wi Bone Densitometer) will be used to measure total body fat, trunk fat and lean mass (145). AT Biopsies. Using a standard CHPS protocol, subcutaneous white AT will be collected at 1400h by core needle aspiration through a 4-mm gluteal incision and will be treated with RNA Later (Qiagen), snap frozen and stored at -80°C for subsequent RNA extraction (121,122,146,147). Cognitive Inhibition Test Battery. We will use a computer-based battery of tests to assess three aspects of inhibition that have been linked with overeating and disordered eating behaviors. The Stop Signal Task (SST) (Logan, 1994) is a well-validated measure of response inhibition that has been used in previous studies and obesity (Mole, 2015; Svaldi, 2014). Participants respond to left and right-facing arrows (go signal) on the computer screen. On 25% of trials of the 64-trial task blocks, a stop signal (an 800-Hz, 100-ms, 70-dB tone) is presented indicating that response should be inhibited. The initial stop delay in each block is 250ms and adjusts ±50ms depending on whether the participant successfully inhibits (Logan, 1994). The primary outcome is stop signal reaction time (SSRT), calculated as the mean RT on go-trials minus mean stop delay. The Stroop Test is a well-validated measure of interference control, or the ability to suppress habitual responses when shown a colored word and being asked to press the key associated with the color of the word rather than the word itself (Stroop 1935). Higher interference scores are associated with obesity (Cohen, 2011; Fagundo, 2012)

and disinhibition assessed by a subscale of the TFEQ (Maayan, 2011). The primary outcome is the interference score, which is calculated as RT (incongruent) minus RT (congruent) and measures the ability to suppress a habitual response in favor of an unusual one, controlling for speed of naming. The Delay Discounting Task* (DDT) (Wileyto, 2004) is a measure of impulsivity that has been associated with obesity and eating behavior in numerous studies (Appelhans 2012, Dassen 2018, Klement 2018), In the DDT, participants choose between a smaller reward available immediately and a larger reward available after a longer delay. The immediate reward will be fixed and the magnitude and delay of the larger, later reward will vary from trial to trial. Participants will make 51 choices. The primary outcome will be the participants discount rate, which will be estimated by fitting a logistic regression that assumes a persons decisions are a stochastic function of the difference in subjective value between the two options (Wileyto, 2004). Keeping with standard behavioral findings (Kirby, 2003; Mazur, 1987), we will assume that subjective value (SV) is a hyperbolic function of the reward amount (A) and delay (D): $SV = A/(1+kD)$, where k is the participants discount rate. Larger values of k indicate a greater degree of discounting future rewards. A composite measure of cognitive inhibition will be calculated to be used in our analyses by averaging individual z-scores derived from the SST, Stroop interference score, and k-value. To eliminate time-of-day effects, we will administer the cognitive inhibition in the afternoon of the first day of each of the inpatient visits. These tasks take approximately 25 minutes to complete. Frequently-Sampled Intravenous Glucose Tolerance (FSIGT) Test. The FSIGT will occur in the CHPS on the second morning at 0800h (with glucose injection) after an 11h overnight fast (participants will stop eating by 2100h the night before). Following baseline blood sampling of 4mL at -15, -10 and -5 minutes, 0.3 g/kg of 50% glucose will be injected over 1 min starting at t=0 and 0.03 U/kg of insulin (1 U/1 ml solution) will be injected over 30 sec starting at t = 20 min. Additional 4mL blood samples will be collected at t=2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22, 25, 30, 40, 50, 70, 100, 140, and 180 min after the injection of glucose (123,148). Plasma glucose will be determined in duplicate by the glucose oxidase method using an automated glucose analyzer (Hitachi 912l; Roche Diagnostic Systems). Plasma insulin will be measured in duplicate by double-antibody radioimmunoassays (Millipore, Billerica, MA) at the Penn Diabetes Research Center Radioimmunoassay & Biomarkers Core. Free fatty acids (FFA) will be measured in duplicate using enzymatic colorimetrics (Wako Chemicals, Richmond, VA). Each participants samples from all 4 FSIGT tests will be assayed simultaneously. The FSIGT parameters, acute insulin response to glucose (AIRglu), insulin sensitivity (SI), glucose effectiveness (SG), and disposition index (DI), will be derived from Bergmans minimal model using MINMOD Millennium software (version 6.02; 149). AIRglu, a measure of first-phase insulin secretion, is calculated as the incremental area under the curve (AUC) for insulin between 0 and 10 min after injection of glucose. SI, a measure of the capacity of insulin to promote glucose disposal, is modeled from the relationship of incremental insulin values over baseline to glucose disappearance. SG, a measure of the capacity of glucose to mediate its own disposal, is modeled from the effect of basal insulin levels on glucose kinetics. DI is a composite measure of -cell function that accounts for the relationship between insulin secretion and sensitivity by the product of AIRglu and SI, providing an assessment of the appropriateness of -cell compensation for changes in insulin sensitivity. The FFA profile during the FSIGT will be analyzed according to the model of Sumner et al. (150), in which 3 phases of FFA dynamics have been described during the 180 min after the injection of glucose; an extension of basal levels until the initial sustained decrease in FFA; the suppression of FFA until nadir evaluated by the fractional disposal rate; and the time from nadir to return to basal levels as also reported by our group (123). Transcriptomics of Blood and AT. The Wistar Institute Genomics Facility will process blood and AT for transcriptomics using standard protocols in place. Total RNA will be isolated from whole blood collected in PAXgene Blood RNA tubes (see section C.6b.) using PAXgene Blood miRNA kits (Qiagen), and Total RNA will be isolated from AT using Direct-zol RNA Miniprep kits (Zymo Research). Extracted RNA will undergo extensive quality control with the Agilent Bioanalyzer 2100 (Agilent), and Qubit fluorometric quantitation (Life Technologies) will be used to obtain accurate RNA concentrations before library preparation. QuantSeq 3mRNA-Seq Library Preparation Kits (Lexogen) will be used for library construction and KAPA Library Quantification Kits will be used for qPCR-based quantification of libraries. Library quality control will be performed with the Agilent Technology 2100 Bioanalyzer using a High Sensitivity DNA chip. Libraries will be pooled and then undergo high-throughput sequencing using the Illumina NextSeq 500.

The following documents are currently attached to this item:

There are no documents attached for this item.

Deception
Does your project use deception?
No

International Research
Are you conducting research outside of the United States?
No

Analysis Plan
Overview Our study proposes a within-subjects cross-over design. With a pre- and post- measurement for both eating conditions per participant for the outcomes, this design allows for comparisons within subject over time and between conditions. We will design our subject randomization on a two-treatment Latin Square design randomized for available subjects. This will allow us to include effects from the order of treatment (delayed eating first vs. daytime eating first) in the mixed effects model as a treatment order effect. General Approach The first phase of the analysis will be descriptive. The sample will be characterized by demographic variables (e.g., age, gender, race and ethnicity), health status (e.g., BMI and medical status) and behaviors (e.g., physical activity, dietary habits, sleep and eating timing). Categorical variables will be summarized by frequencies, while continuous variables will be summarized by the mean and 95% confidence intervals, median, standard deviation and range. The predictor of interest for each aim will be the eating condition (daytime vs. delayed). An analysis of data distributional properties will be performed to determine if variance stabilizing or normalizing transformations should be applied to the outcome measures as a function of eating condition. The second phase of the analysis will consist of model building and interpretation. For within-group outcomes, all available data will be used to estimate changes over time. Other fixed covariates in the models will account for chance imbalances (not balanced by randomization, such as subject) at the start of the study. Group-by-time interaction terms will estimate the differences in changes over time between the daytime and delayed eating condition on each continuous outcome of interest. Further analyses will adjust for departures from randomization related to dropout or suboptimal adherence (e.g., eating or sleeping outside the assigned time window, eating non-provided meals, exercising more than allowed). Thus, separate models will be generated for each of the outcome measures, with each outcome measure regressed on eating condition assignment, along with baseline outcomes and any other covariates deemed prognostic in preliminary analyses, including adherence measures such as sleep and eating timing, and exercise. Since there are two orders (daytime-delayed or delayed-daytime), clustering of participants will be modeled using a fixed covariate to indicate order. Assessments from individuals repeated over time are more alike than expected by chance and failure to consider the non-independence of assessments will yield misleading results due to the underestimation of variance. Mixed effects models with random intercepts and slopes for participants will account for the within-subjects correlation. Thus, we expect the linear mixed models to account for fixed treatment and order effects, and random subject effects. Missing data are sometimes handled by removing individuals with incomplete data from the analyses, a strategy that may potentially produce biased estimates such that those with complete data do not represent the original sample. The proposed analytic method (mixed effects models) assumes that data are missing at random (i.e., missing data are dependent only on observed, past values of covariates and outcomes), and these models use all available data. This approach will not account for informative dropout, where dropout occurs for reasons related to unobserved outcomes. Thus, sensitivity analyses will be run to estimate the potential impact of missing data on overall response to the daytime and delayed eating phases assuming that those with no measurements fared worse than those who remained in the study (151). Bias from non-compliance may also occur if participants do not adhere to the prescribed eating conditions. Non-compliance may be associated with outcomes and act as a confounder. To address this potential issue and estimate the effect of the eating conditions in the presence of full compliance, we will implement instrumental variable methods (152,153). All analyses for these 3 aims will be performed in the latest versions of the analytical software programs, SAS (v 9.3), Stata (v 13) and R (v 3.3.3). Approach by Specific Aim Specific Aim 1. The goal of this aim is to compare the daytime vs. delayed eating schedule on body mass (weight), adiposity, and energy homeostasis outcomes. We will fit separate linear mixed effect models for each outcome as described above. Baseline covariates that are found to be associated with missing data or unbalanced after randomization will be added to the models. Appropriate contrasts will be estimated with 95% CIs to quantify the difference between the daytime vs. delayed schedules in the reduction of outcomes. Specific Aim 2. The goal of this aim is to compare the daytime vs. delayed eating schedule on the phase changes in the circadian ghrelin and leptin rhythms and the phase and cycling of gene expression profiles from blood. Analysis of the circadian phase of hormonal and

amplitude and phase of expression data will use linear mixed effects cosinor analysis (154), which accounts for systematic inter-individual differences. We will fit separate linear mixed effect models for ghrelin and leptin phases as described above. For transcriptomics, gene expression count data will be normalized in R using the TMM function in edgeR (155) and voom in the limma package (156). We will use linear mixed effect models against RNA-seq depth count data to test the differences in genes between the daytime and delayed conditions. Mixed model analysis will be done on the normalized RNA count data to adjust for effects such as condition order and random subject effects using the lme4 package (157) in the R statistical environment version R-3.2.3. P-values for each test will be generated from the lmer results using the Anova function in the R car package (158). Coefficients of the lme analyses will represent the log2 fold change between the two conditions tested (change in daytime vs. change in delayed). We will use a p-value cutoff adjusted by false discovery rate of 0.05 to detect all RNA changes of statistical significance comprehensively. Pathway analysis will be run in various functional enrichment analysis tools, e.g., Metacore (Thomson Reuters) and Ingenuity Pathway Analysis software (Qiagen). We also will cluster genes by phase for each of the 4h sample bins and compare them with circadian rhythm hormone phase profiles using correlational analysis to identify how different blood markers are related as a function of eating condition; this may allow for identification of additional genes implicated in timed eating. Each phase cluster can also be analyzed for statistical significance of enrichment against a number of biological pathways and gene sets. Specific Aim 3. The goal of this aim is to compare the daytime vs. delayed eating schedule on insulin sensitivity and free fatty acid dynamics and on AT gene expression. Changes in insulin sensitivity and free fatty acid dynamics by condition will be compared with linear mixed-effects models. Statistical analysis of gene expression data will be conducted as described for Specific Aim 2. As AT and blood will be collected on the same day, we will also compare pre-post changes in AT and blood expression using meta-analyses, to identify how different tissues relate to each other as a function of eating condition. This will also allow indirect comparison of hormonal data with AT gene expression. Finally, we will correlate AT gene expression with fasting lipid and glucose levels.

The following documents are currently attached to this item:

There are no documents attached for this item.

Data confidentiality

- x **Paper-based records will be kept in a secure location and only be accessible to personnel involved in the study.**
- x **Computer-based files will only be made available to personnel involved in the study through the use of access privileges and passwords.**
- x **Prior to access to any study-related information, personnel will be required to sign statements agreeing to protect the security and confidentiality of identifiable information.**
- x **Wherever feasible, identifiers will be removed from study-related information.**

A Certificate of Confidentiality will be obtained, because the research could place the subject at risk of criminal or civil liability or cause damage to the subject's financial standing, employability, or liability.

A waiver of documentation of consent is being requested, because the only link between the subject and the study would be the consent document and the primary risk is a breach of confidentiality. (This is not an option for FDA-regulated research.)

Precautions are in place to ensure the data is secure by using passwords and encryption, because the research involves web-based surveys.

Audio and/or video recordings will be transcribed and then destroyed to eliminate audible identification of subjects.

Subject Confidentiality

Subject confidentiality will be maintained throughout the study according to applicable guidelines, regulations and IRB requirements. All laboratory samples, study clinical data, and reports of results will de-identify individual subjects. Subjects will be identified by initials, date of birth, gender and subject number only for use in data collection. Published data will provide subject numbers only if needed for clarity of presentation (e.g., in individual event listings). The study will be conducted in accordance with the Declaration of Helsinki. The study will be conducted in accordance with the ICH GCP

guidelines. The sponsor-investigator will comply with all applicable regulatory and legal requirements, ICH GCP guidelines, and the Declaration of Helsinki in obtaining and documenting the informed consent. All protected health information (PHI) will be maintained using a network drive and/or devices secured and managed by University of Pennsylvania. Additionally, if necessary, PHI will also be managed on a University approved third-party computing environment. Should PHI need to be transmitted, a University of Pennsylvania-approved encrypted portable device or University of Pennsylvania-approved secure encrypted file transfer solution will be utilized. The subject and any biological material obtained from the subject will be identified by subject ID and trial identification number. Appropriate measures such as encryption or leaving out certain identifiers will be enforced to protect the identity of subjects.

Sensitive Research Information*
Does this research involve collection of sensitive information about the subjects that should be excluded from the electronic medical record?
No

Subject Privacy
Privacy refers to the person's desire to control access of others to themselves. Privacy concerns people, whereas confidentiality concerns data. Describe the strategies to protect privacy giving consideration to the following: The degree to which privacy can be expected in the proposed research and the safeguards that will be put into place to respect those boundaries. The methods used to identify and contact potential participants. The settings in which an individual will be interacting with an investigator. The privacy guidelines developed by relevant professions, professional associations and scholarly disciplines (e.g., psychiatry, genetic counseling, oral history, anthropology, psychology).
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: Protected health information (PHI) 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 research subjects to revoke their authorization for use of their PHI View of PHI will be limited to individuals at the University of Pennsylvania directly involved in the study. The company donating the study product will not have access to PHI. All electronic PHI will be maintained by using an institutionally secured and managed network drive, institutionally secured and managed devices, and institutionally approved third-party computing environments. Should PHI need to be transferred, it will be done so through the use of a Penn-approved encrypted portable drive or a Penn-approved secure encrypted file transfer solution. In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects who have revoked authorization to collect or use PHI, attempts will be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period. Where possible, data will be entered directly into our password protected database, REDCap. All data pertaining to the study will be saved on the Center for Weight and Eating Disorders password-protected server. Paper copies of informed consent, questionnaires, interviews, lab results, and any correspondence will be kept in the case record in locked offices.

Data Disclosure
Will the data be disclosed to anyone who is not listed under Personnel?
No

Data Protection*

x	**Name**
x	**Street address, city, county, precinct, zip code, and equivalent geocodes**
x	**All elements of dates (except year) for dates directly related to an individual and all ages over 89**
x	**Telephone and fax number**
x	**Electronic mail addresses**
x	**Social security numbers**
x	**Medical record numbers**
	Health plan ID numbers
	Account numbers
	Certificate/license numbers
	Vehicle identifiers and serial numbers, including license plate numbers
	Device identifiers/serial numbers
	Web addresses (URLs)
	Internet IP addresses
	Biometric identifiers, incl. finger and voice prints
	Full face photographic images and any comparable images
	Any other unique identifying number, characteristic, or code
	None

Does your research request both a waiver of HIPAA authorization for collection of patient information and involve providing Protected Health Information ("PHI") that is classified as a "limited data set" (city/town/state/zip code, dates except year, ages less than 90 or aggregate report for over 90) to a recipient outside of the University of Pennsylvania covered entity?
No

Tissue Specimens Obtained as Part of Research*
Are Tissue Specimens being obtained for research?
Yes

Tissue Specimens - Collected during regular care*
Will tissue specimens be collected during regulator clinical care (for treatment or diagnosis)?
No

Tissue Specimens - otherwise discarded*
Would specimens otherwise be discarded?
No

Tissue Specimens - publicly available*
Will tissue specimens be publicly available?
No

Tissue Specimens - Collected as part of research protocol*
Will tissue specimens be collected as part of the research protocol?
Yes

Tissue Specimens - Banking of blood, tissue etc. for future use*
Does research involve banking of blood, tissue, etc. for future use?
Yes

Genetic testing
If genetic testing is involved, describe the nature of the tests, including if the testing is predicative or exploratory in nature. If predictive, please describe plan for disclosing results to subjects and provision

of genetic counseling. Describe how subject confidentiality will be protected Note: If no genetic testing is to be obtained, write: "Not applicable."
Confidentiality will be protected. Genetic analyses will be completed by other laboratories without access to identifying information (i.e., they will have identification code numbers only). Subjects' identities will be protected during and after genetics testing with the same high standards as the rest of their data. We will be conducting exploratory testing targeting genes in the areas of metabolism, sleep and circadian (24-hour) rhythms.

Consent

1. Consent Process

Overview
Study site staff will be obtaining consent by providing a copy and reviewing the consent with all subjects who may be eligible for the current studyl. The consent process will occur at the Center for Weight and Eating Disorders located at 3535 Market Street, Philadelphia, PA 19104. Study staff will explain that the subject can choose whether or not he or she would like to participate. Study staff will then go over the consent if the subject decides to participate. Study staff will inform the participant of their right to withdrawal from the study at any point in time. Possible risks and benefits of participating in the trial will be discussed during the informed consent process. If subject decides to participate, he or she will be asked to sign the consent form.

Children and Adolescents
Not applicable.

Adult Subjects Not Competent to Give Consent
Not applicable.

2. Waiver of Consent

Waiver or Alteration of Informed Consent*
No Waiver Requested

Minimal Risk*

Impact on Subject Rights and Welfare*

Waiver Essential to Research*

Additional Information to Subjects

Written Statement of Research*
No

If no written statement will be provided, please provide justificiation

The following documents are currently attached to this item:

There are no documents attached for this item.

Risk / Benefit

Potential Study Risks
During this study you will be asked questions of a personal nature, such as your weight, age, eating and physical activity habits, and mood. You may experience some discomfort when answering these questions. You may not like all of the foods that we give you which may cause some discomfort.

Additionally, the eating schedule may be difficult to manage at times, and this may cause you some inconvenience. You will experience the inconvenience of wearing the wrist activity recorder and keeping records of your eating behaviors, sleep, and activity throughout the study. For the blood draws, you will experience discomfort on initial insertion of the thin needles (introducer) into a vein on your arm and hand. Bruising may develop at the sites of the needle insertions. Dizziness or fainting is a remote possibility. Local clots may form, and infections may occur, but these are rare. The amount of blood drawn will not significantly reduce your blood volume, although there may be a small decrease in your red blood cell concentration (hematocrit). The total volume of blood drawn will be less than 50 teaspoonfuls, and this loss is readily restored. Occasionally, mild discomfort may occur from a catheter in your vein. If this happens, we will either change its position or remove it entirely, asking your permission before reinserting it. There may be some bruising when the catheters are removed. You will be asked to apply pressure to the site of each catheter for 10 minutes after its removal. There may be a small scar at the catheter sites that will disappear over the course of several months. This research involves exposure to radiation from the DEXA scans. Therefore you will receive a radiation dose. This radiation dose is not necessary for your medical care and will occur only as a result of your participation in the study. At doses much higher than you will receive, radiation is known to increase the risk of developing cancer after many years. At the doses you will receive, it is very unlikely that you will see any effects from the radiation dose. Risks associated with the fat biopsy include bleeding, bruising, infection, possible scarring, and pain. These risks will be reduced by using a trained physician/ nurse practitioner to perform the procedures, local anesthesia to reduce pain, and careful monitoring of the site where the fat cells were taken. For the FSIGT test, a known risk is low blood sugar. We will administer insulin 20 minutes after the glucose is given to you to ensure that your blood sugar returns to normal. As insulin lowers blood glucose, there is a possibility that the insulin will make your blood glucose go too low. Low blood sugar results in sweating, shaking, and mental confusion. We will monitor your blood sugar and give you food or glucose directly into your blood if your blood sugar goes too low. The study involves restraining you from eating for a period of at least 10 hours (overnight) while in the hospital, which may be uncomfortable. During the assessment visits you will be staying in a dimly lit room and will be unable to bring any electronics that will emit any extra light, such as your cell phone. This may be an inconvenience. If you are currently pregnant or become pregnant while in the research study, it is important that you inform the investigator because you will not be able to participate. If you are able to become pregnant, you must be given a pregnancy test during the screening period and at each assessment visit. You are asked to use a medically accepted method of birth control (such as condoms, birth control pills or patches, IUDs, etc.) while you participate in the study. The research may involve risks that are currently unforeseeable. If you are injured, you should inform the treating physician that you are in a research study.

Potential Study Benefits
There are no expected benefits from being in this research study. Study participation may contribute important data for understanding how eating at certain times of day affects weight management and how the body processes and stores food.

Alternatives to Participation (optional)
The only alternative to participation is to choose not to participate.

Data and Safety Monitoring
Please see the attached data safety monitoring plan.

The following documents are currently attached to this item:

There are no documents attached for this item.

Risk / Benefit Assessment
The study is considered minimal risk because the risks anticipated from the proposed research study are no greater than those ordinarily encountered during the performance of routine tests (see risks in "potential study risks" section) or in daily life. Participation may contribute important data for understanding how eating at certain times of day affects weight management and how the body processes and stores food.

General Attachments

The following documents are currently attached to this item:

Additional forms (lo_hippa.pdf)

Additional forms (lo_gcp.pdf)

Additional forms (lo_citi.pdf)

Cover Letter (coverletter_irbcoverletter_modification6.10.21.docx)

Additional forms (documentsuploadedforreviewmod6.10.21.docx)