Cover Page for Trial Protocol

Official Title	Effect of Time-restricted Eating on Catecholamine-sensitivity of Adipose Tissue in Obese Adults
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UCSD Human Research Protections Program

New Biomedical Application
RESEARCH PLAN

Instructions for completing the Research Plan are available on the HRPP website.
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General Instructions: Enter a response for all topic headings.
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Version date: 9/30/2013

1. PROJECT TITLE

Effect of time-restricted eating on catecholamine-sensitivity of adipose tissue in obese adults

2. PRINCIPAL INVESTIGATOR

Michael J. Wilkinson, MD FACC (UC San Diego Division of Cardiovascular Medicine)

3. FACILITIES

UCSD Hospitals and Clinics,
Altman Clinical and Translational Research Institute (ACTRI)
Salk Institute for Biological Studies

4. ESTIMATED DURATION OF THE STUDY

2 years

5. LAY LANGUAGE SUMMARY OR SYNOPSIS (no more than one paragraph)

Our prior research has demonstrated that time restricted eating (TRE) is a lifestyle change that helps those with excess body fat lose weight and lowers blood pressure and cholesterol. In a randomized controlled trial, we intend to uncover the mechanism through which TRE benefits those with obesity who habitually eat for more than 14 hours each day by studying its effects on an aspect of fat metabolism known as catecholamine sensitivity (response to "fight or flight" hormones). Patients will be randomly assigned to a control group of behavioral nutrition counseling (standard of care) or the intervention group of behavioral nutrition counseling with the addition of adopting a 10 h eating window for 12 weeks (TRE). We will obtain fat samples from under the skin using a needle, and will evaluate the impact of TRE on aspects of metabolism in fat tissue that are related to the breakdown of fat which is important for weight loss. One aspect of fat metabolism, catecholamine sensitivity, will be tested by exposing fat tissue samples to catecholamine hormones in the laboratory. Changes in weight, percent body fat, blood pressure, cholesterol levels, and blood sugar levels will also be measured. These assessments will be made at baseline and at the end of the 12-week intervention period to assess changes. Food and drink intake, activity, and sleep will be monitored with the smartphone myCircadianClock application ("mCC app") throughout the study.

6. SPECIFIC AIMS

Safe, effective dietary interventions that patients can adhere to lifelong are needed to help reduce the worldwide burden of obesity and related diseases, especially type 2 diabetes mellitus (T2DM). Obesity is a key driver of cardiovascular disease (CVD) and intricately linked to T2DM. Insulin resistance has been historically a key target for improving outcomes in obese patients. However, recent studies have made it clear that resistance to other hormones, notably catecholamines, is an under-appreciated attribute of obesity, and possibly a key factor hampering weight loss efforts and negatively impacting cardiometabolic health and is thus a target for lifestyle and therapeutic intervention.

Time-restricted eating (TRE) is a dietary intervention based on circadian rhythm biology that promotes weight loss and improves metabolic health in animal models. Few studies of TRE have been performed in humans, but findings suggest TRE may be a powerful lifestyle intervention to promote cardiometabolic health. We have recently demonstrated that in 19 patients with obesity (mean body mass index (BMI) 33 kg/m^2) and metabolic syndrome, adherence to TRE for 10-hours/day (14 hours of fasting/night) for 3 months promotes weight loss and loss of visceral fat, along with lower blood pressure, total cholesterol, low-density lipoprotein cholesterol (LDL-C), and non-high-density lipoprotein cholesterol (non-HDL-C). Importantly, most participants were already taking statins and blood pressure lowering medications, and therefore these improvements were seen in the setting of optimal, standard of care background medical therapy and relatively low atherogenic lipid levels at baseline. These improvements were independent of caloric intake and physical activity, and long-term adherence to TRE for at least ~16 months was feasible (Wilkinson et al., *Cell Metabolism*).[1]

Understanding the full clinical impact of TRE requires elucidation of its effects at a mechanistic level in humans in order to understand the drivers of improved cardiometabolic health. This study will provide insight into the effects of TRE on lipolysis and loss of fat mass and may help us to predict whether TRE may be synergistic with other lifestyle and pharmacologic interventions to improve metabolism. In mouse models, TRE has favorable effects on adipose tissue, including greater fat catabolism (lipolysis, less stored triglyceride, more lipid oxidation) and less inflammation.[2] The overarching goal of this study is to establish the effects of TRE on the metabolic activity of adipose tissue in patients with obesity. We will accomplish this by measuring the catecholamine sensitivity of adipose tissue. Catecholamines are the key drivers of lipolysis in adipose tissue, and work by binding to β2 and β3 adrenergic receptors (ADRB2 and 3) on the surface of adipocytes. While ADRB2 is expressed in many tissues, ADRB3 is relatively specific for adipocytes. Due to chronic inflammation, expression of adipocyte ADRB3 is down-regulated in obesity, which reduces lipolysis and lipid oxidation, increases storage of triglycerides and impairs loss of fat. Weight re-gain frequently follows successful weight-loss, partly because chronic inflammation in obesity causes persistent down-regulation of ADRB3, leaving adipose metabolically inflexible and in a state of energy preservation instead of burning energy to promote weight loss. Because TRE has favorable effects on adipose tissue metabolism in mice (less stored fat and less inflammation), we hypothesize that TRE improves metabolism in adipose tissue by improving catecholamine sensitivity. Further, because inflammation reduces catecholamine sensitivity, if improved catecholamine sensitivity occurs with TRE, this might be due to less inflammation in adipocytes with TRE. I will test these hypotheses according to the following specific aims:

Specific Aim 1: Examine the effects of TRE on catecholamine sensitivity of adipose tissue.
Hypothesis: TRE will improve catecholamine sensitivity in adipose tissue, measured by 1) an *in vivo* increase in β3 adrenergic receptor expression (ADRB3) and 2) an increase in the *ex vivo* response of adipose tissue to direct stimulation with catecholamines.

- ADRB3 receptor expression will be measured in adipose from human subcutaneous fat biopsy by isolating mRNA and performing qPCR, and by measuring ADRB3 protein levels by Western blot.
- Gene expression related to the down-stream effects of catecholamine stimulation of adipocytes will be evaluated by RNA-sequencing (RNA-seq).
- The sensitivity of adipose tissue to catecholamines will be measured *ex vivo* by stimulating tissue with isoproterenol and measuring the release of the products of lipolysis (glycerol and free fatty acids (FFAs)).

If TRE improves catecholamine-sensitivity in adipose tissue, in vivo ADRB3 *mRNA and protein levels should increase and more glycerol and FFAs will be released during* ex vivo *stimulation of tissue with isoproterenol.*

Specific Aim 2: Examine the effects of TRE on inflammation in adipose tissue.
Hypothesis: TRE will reduce inflammation in adipose tissue, measured by a decrease in TNFα and IL1β.
Inflammation will be measured by examining gene expression, using mRNA and qPCR for *TNF* and *IL1B*, as well as by examining gene expression in other parts of the inflammatory pathway through RNA-seq. *If TRE improves inflammation in adipose tissue,* TNF and IL1b *mRNA levels should decrease, representing a potential underlying mechanism by which TRE may improve catecholamine-sensitivity in adipose tissue.*

Specific Aim 3: Examine the effects of TRE on *in vivo* lipid metabolism in adipose tissue.
Hypothesis: Lipolysis will increase in adipose tissue during TRE, which may be reflected in higher plasma FFA levels. Lipolysis increases in adipose tissue when ADRB3 receptor expression is increased, and we hypothesize that TRE will promote ADRB3-mediated catabolism (lipolysis and lipid oxidation) in adipose tissue (Specific Aim 1). *In vivo,* plasma FFA levels are determined by multiple factors (lipolysis in adipose tissue increases plasma FFAs, while FFA oxidation in adipose tissue and uptake of FFAs by the liver may decrease plasma FFAs). *However, if TRE promotes significantly enhanced lipolysis in adipocytes, we expect that FFA levels in plasma will increase.*

Exploratory Aim 1: Examine total body changes in fat distribution and fat mass during TRE.
Hypothesis: Using dual-energy X-ray absorptiometry (DEXA), we expect that with TRE we will observe healthier fat distribution and a reduction in total body fat mass.

Exploratory Aim 2: Examine the effects of TRE on multiple cardiometabolic biomarkers.

Hypothesis: TRE will improve multiple cardiometabolic biomarkers, and will promote lower blood pressure, lower atherogenic lipoprotein levels, improved glucose metabolism (lower fasting glucose, insulin, and mean glucose by continuous glucose monitoring), lower systemic inflammation, and favorable changes in adipokines (lower leptin, increased adiponectin).

7. BACKGROUND AND SIGNIFICANCE

There is a worldwide epidemic of obesity and related cardiometabolic diseases, including T2DM, and lifestyle interventions are needed

Obesity is a growing public health problem, with recent data showing a prevalence of obesity (defined as BMI ≥ 30 kg/m^2) in the United States of 39.6% among adults. Obesity is associated with a shorter lifespan and higher BMI is associated with a higher risk of death due to CVD.[3] Lifestyle change is first line therapy for obesity.[4] Safe, effective, sustainable methods for lifestyle change to address obesity and promote metabolic and cardiovascular health are needed. TRE could be a powerful lifestyle tool for addressing the obesity epidemic and potentially reducing risk for T2DM, but more data are needed in humans including elucidation of the effects of TRE on adipose tissue in obesity.

Obesity impairs normal energy expenditure in adipose tissue through catecholamine resistance

A key driver of lipolysis in adipose tissue is the binding of catecholamines to the β3 adrenergic receptor (ADRB3) on adipocytes (**Figure 1**). The regulation of ADRB3 expression in adipocytes is complex, and reduced expression of ADRB3 can occur for several reasons. There are clear links between the chronic inflammatory state in obese adipose tissue and reduced ADRB3 expression. Work from the lab of my mentor, Dr. Alan Saltiel, has explored this pathway. Under normal conditions, binding of catecholamines to ADRB3 on adipocytes leads to an increase in cAMP, which promotes activation of protein kinase A (PKA) and thereby increases lipolysis and lipid oxidation through the phosphorylation and activation of hormone sensitive lipase (HSL). Thermogenesis also increases, driven by a PKA-dependent induction of UCP1.[5,6]

This catabolic system breaks down when expression of ADRB3 is reduced, such as in obesity. Due to the low-grade inflammatory state in obese adipose tissue, ADRB3 expression is reduced because of an increase in the activity of noncanonical kinases IKKε and TBK1 (part of the NFkB inflammatory pathway) and associated down-stream PDE3B activity, which involves lower cAMP levels. Inflammation, such as increased activity of TNFα, promotes increased activity of the noncanonical kinases IKKε and TBK1, ultimately leading to reduced ADRB3 expression which leads to a reduction in lipolysis.[7] The drug amlexanox is an oral medication which was originally developed to treat asthma, allergic rhinitis, and aphthous ulcers.[8] Interestingly, amlexanox inhibits IKKε and TBK1 and can thereby restore ADRB3 expression and associated lipolysis in adipose tissue in diet-induced obese mice on HFD (**Figure 1**).[7] In obese humans with T2DM and non-alcoholic fatty liver disease (NAFLD) (n=6), amlexanox improves ADRB3 receptor expression, measured as an increase in *ADRB3* RNA in a subset of patients with high levels of baseline adipose tissue inflammation.[9] Thus, the key to promoting catabolism in adipose (lipolysis, lipid oxidation, and ultimately loss of fat mass) may lie in finding ways to promote ADRB3 expression in the face of obesity and inflammation. It is unknown whether a dietary intervention in obese patients, like TRE, could counteract catecholamine resistance in adipocytes, however multiple metabolic benefits of TRE have been observed in mice without a reduction in caloric intake, including protection against the development of excess body fat and less inflammation in adipose tissue.[2]

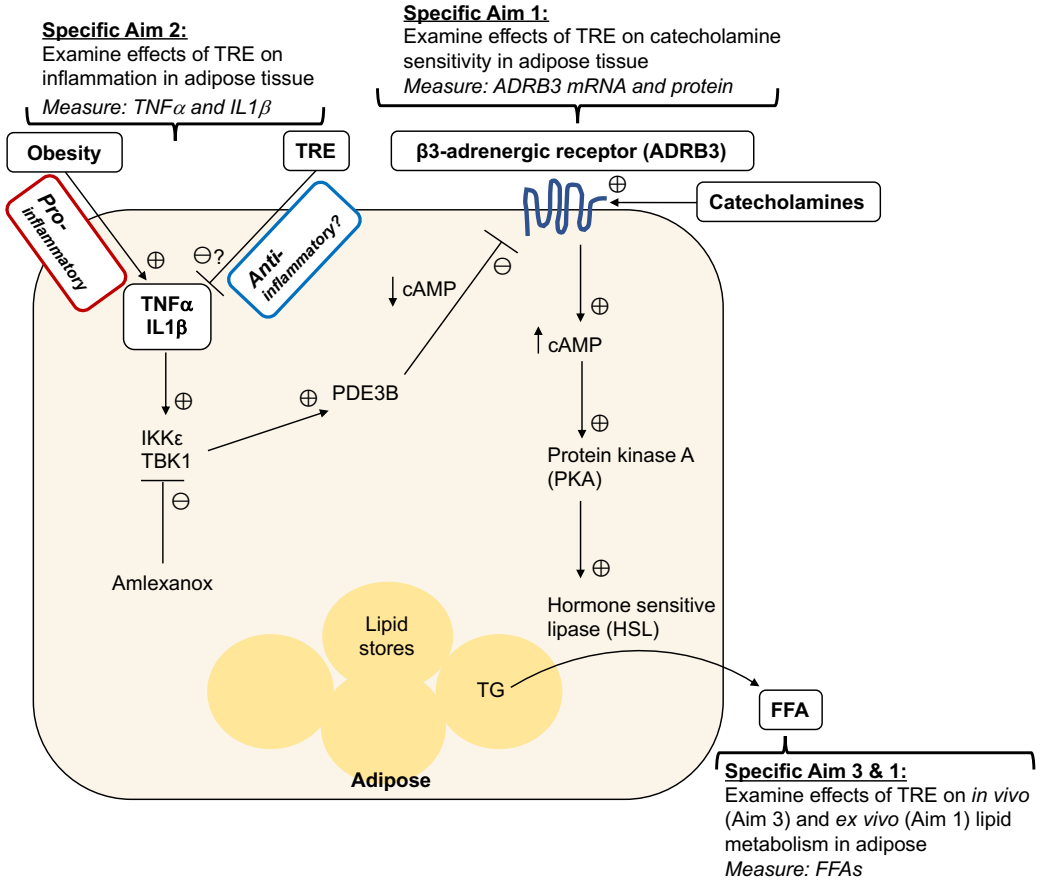

Figure 1. Mechanism of catecholamine-driven catabolism in adipose tissue, including a possible mechanism for improved adipose catecholamine sensitivity with TRE by reducing inflammation. Chronic inflammation in obesity results in reduced expression of the β3-adrenergic receptor, which leads to less downstream catabolism of stored triglyceride by hormone sensitive lipase. Time-restricted eating leads to less inflammation and less stored fat in adipose tissue from animal models of obesity, but the mechanism is unknown and has never been studied in humans.

Time-restricted eating aligns metabolism with the circadian rhythm, promoting beneficial metabolic changes

TRE involves limiting all dietary intake to a "window" during daytime hours; for example, a TRE window of 10-hours might require that all dietary intake other than water occur between 8am and 6pm. This creates a period of 14-hours of fasting per night. My mentor, Dr. Panda, has shown that TRE is both preventative and therapeutic against glucose intolerance in mice and has other beneficial cardiometabolic effects in mice and flies.[2,10,11]

In mice models, without a reduction in caloric intake, TRE protects against the accumulation of excess body fat.[2] Mice on TRE also have smaller lipid droplets in white adipose tissue and are protected against the development of lipid droplets in brown adipose tissue (**Figure 2**).[2] As expected with less body fat, TRE was associated with lower leptin levels, and increased adiponectin. Importantly, TRE was associated with a reduction in mRNA levels of proinflammatory cytokines (including TNFα and IL1β) and chemokines in adipose.[2] Decreased inflammation could contribute to enhanced

β3 receptor expression in adipocytes, as hypothesized in this proposal (**Figure 1**). Such a detailed analysis of metabolism and gene expression in adipose tissue from humans undergoing TRE has never been performed.

Preliminary Studies

Under the mentorship of Dr. Panda and Dr. Pam Taub, I recently helped to complete a pilot study examining the effects of TRE on 19 patients with metabolic syndrome and obesity. Patients reduced dietary intake from a baseline mean daily eating period of ≥ 14 hours to a self-selected 10-hour window for 12-weeks. We observed significant reductions ($p<0.05$) in body weight (mean±SD -3.3±3.20 kg (-3%)), waist circumference (-4.5±6.72 cm (-4%)), BMI (-1.1±0.97 kg/m^2 (-3%)), percent body fat (-1.0±0.91% (-3%)), visceral fat rating (-0.6±0.77 (-3%)), systolic and diastolic blood pressure (-5.1±9.51 mmHg (-4%) and -6.5±7.94 mmHg (-8%), respectively), total cholesterol (-13.2±24.29 mg/dL (-7%)), LDL-C (-11.9±19.01 mg/dL (-11%)), and non-HDL-C (-11.6±22.94 mg/dL (-9%)) (Wilkinson et al., *Cell Metabolism*).[1] Participants with elevated fasting glucose (≥100 mg/dL) and/or glycated hemoglobin (HbA1c) (≥5.7%) at baseline (n=12), had a significant reduction in HbA1c (-0.22±0.32% (3.7%), p=0.04). Improvements were observed despite no change in physical activity (mean change in daily activity counts by Philips Actiwatch -13281.03±29037.07, p=0.069). Most participants were already taking statins and blood pressure lowering medications. There was an 8.62%±14.47% decrease in mean daily caloric intake during intervention (1792.00±578.08 calories) compared to baseline (1990.59±649.89 calories; p=0.007). Our analysis suggests that the improvements we observed in cardiometabolic end-points with TRE were independent of changes in weight. Our data suggest that TRE represents a feasible and effective circadian rhythm based behavioral intervention to treat metabolic syndrome among those already receiving routine medical care including pharmacotherapy. If TRE has favorable effects on catecholamine sensitivity of adipose tissue, then it is likely to represent a safe, feasible dietary intervention for promoting sustained, long-term weight loss and improved metabolic health.

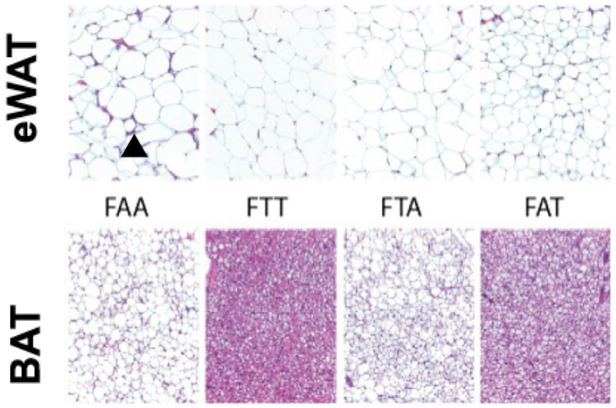

Figure 2. Time-restricted eating (TRE) results in healthier adipose tissue, characterized by smaller lipid droplets in white (eWAT) and brown (BAT) adipose tissue on H&E staining. FAA: mice fed high-fat diet (HFD) ad lib. FTT: HFD with TRE. FTA: HFD with TRE followed by ad lib feeding. FAT: HFD with ad lib feeding followed by TRE. Arrowhead refers to crown-like structures (dead adipocytes). Chaix et al. 2014

8. PROGRESS REPORT

None.

9. RESEARCH DESIGN AND METHODS

Summary

We will enroll up to 120 participants (accounting for a 15% attrition after enrollment, this will provide a sample size of 102 participants) ≥ 18 years with obesity (BMI ≥ 30 kg/m^2) (without T2DM) and a baseline eating duration of ≥ 14 hours/day who will be randomized 1:1 to TRE (10-hours/day) vs control (standard of care diet). Participants will be recruited from the cardiovascular and internal medicine clinics at UCSD. In addition, we will utilize the bioinformatics report generated from EPIC to assist in recruitment. Participants will be followed for 3 months and use the myCircadianClock (mCC) smartphone app (developed by Dr. Panda) to log dietary intake. At the beginning and end of the study, participants will undergo adipose tissue biopsy after a 14-hour overnight fast. Gene expression will be measured in adipose tissue samples through qPCR, RNA-seq and Western blot. Catecholamine sensitivity will be tested by exposing adipose tissue samples to isoproterenol *ex vivo* and measuring the production of glycerol and FFAs. Anthropometric measures, DEXA, and plasma biomarkers will also be measured.

Study visits and procedures

The study will consist of 4 or 5 in person visits over 14 weeks, as well as a 1-2-week pre-screening period which will provide a total study length of 15-16 weeks. After the 1-2-week pre-screening period where the study team determine preliminary eligibility through the participant's logging on the mCC app, the participant will undergo 2 weeks of screening/baseline and 12 weeks of intervention (**Figure 3**). Prior to the pre-screening period, the participant will be

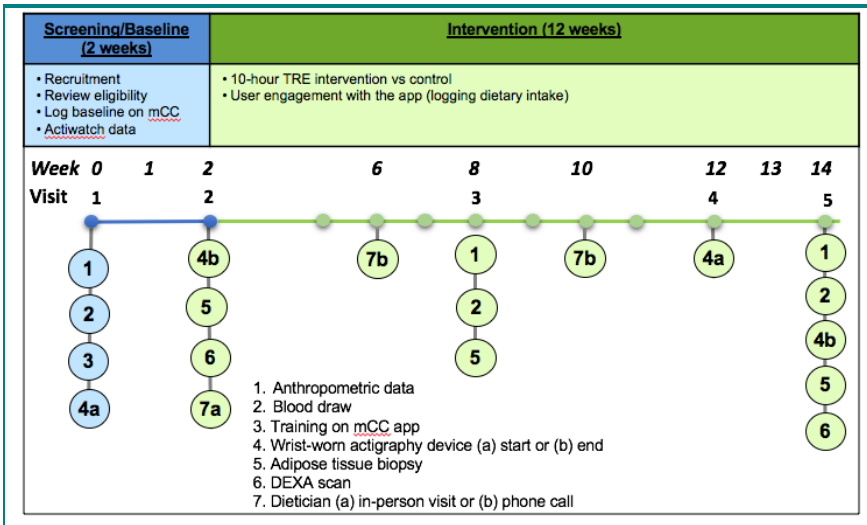

Figure 3. Study design. Obese participants will undergo 2 weeks of screening/baseline, randomization to TRE versus standard of care, and 12 weeks of intervention. mCC, myCircadianClock smartphone app, CGM, continuous glucose monitor, TRE, time-restricted eating, DEXA, dual-energy X-ray absorptiometry.

provided with the ICF via email. If the participant acknowledges their consent via email, they will enter the pre-screening period and be instructed on how to download the mCC app and log their food and beverage intake over 1-2 weeks. Only if the participant's eating window is deemed as qualifying, defined as an eating window of 14 hours or more, they will be eligible to proceed with the study and their visit 1. At visit 1 (day 1: Screening/Baseline) participants will provide written informed consent, and baseline anthropometric measurements and fasting blood tests will be obtained by standard venipuncture: comprehensive metabolic panel, complete blood count, thyroid stimulating hormone, hemoglobin A1c (HbA1c), lipoprofile by nuclear magnetic resonance (which includes standard lipid panel, lipoprotein particle numbers, and lipoprotein subfractions), coagulation parameters (INR/PT and PTT), insulin, hs-CRP, TNF-alpha, IL1-beta, leptin, adiponectin, and plasma free fatty acids. Comprehensive blood work will be done to ensure that there are no other confounders such as renal dysfunction, anemia, hypo- or hyperthyroidism, which could influence results of the study (**See Table for Inclusion and Exclusion Criteria (below under Human Subjects**)). For women of child-bearing age, a urine pregnancy test will be performed and if positive will result in study exclusion. Using the mCC smartphone app, participants take a photo of all food and beverage they are about to consume, and the image is time-stamped and uploaded to a secure server. Study coordinators are able to monitor participant data to assess adherence with logging during the trial. A continuous glucose monitor (CGM) will be placed on the back of the upper arm, and participants will be given a wrist-worn actigraphy device to wear. Participants will be asked to use the mCC app to log all dietary intake for 2 weeks. Participants who meet inclusion criteria, including a baseline eating interval ≥ 14 hours during the screening period, will be randomized to TRE or standard of care. At visit 2, participants will present in the fasting state and abdominal fat biopsy will be performed[9]: after anesthetizing skin of the abdomen with 1% lidocaine injection, a 16-gauge needle or aspiration cannula connected to a 20 mL syringe is used to obtain subcutaneous adipose tissue (~0.6 g of tissue). A DEXA scan will be performed. Additional blood work may be obtained at this visit, approximately 18ml (4 teaspoons) will be collected. All participants meet with a dietician to receive behavioral nutritional counseling. Participants in the TRE arm will self-select a 10-hour eating window. TRE is the only intervention, though all patients will be educated about the Mediterranean diet and will be encouraged to implement it in their daily lives. The control group will be given standard health and wellness guidelines and they will be advised to continue their habitual eating pattern spread over >14h. Their mCC app will be programmed so that they can visualize their own caloric intake spread over the wakeful hours. The intervention period will run 12 weeks. All participants will receive a phone call from a dietician after 6 and 10 weeks of study enrollment to reinforce healthy dietary practices. At visit 3 (week 8) participants will return at mid-intervention and will undergo a blood draw, adipose tissue biopsy, and anthropometric measures. At visit 4 (week 12), participants will have a CGM and wrist-worn actigraphy device placed to wear for 2 weeks. At visit 5 (week 14: Study end), CGMs and wrist-worn actigraphy devices will be returned, final anthropometric measurements, fasting blood work, DEXA and fat biopsy will be performed. Participants that successfully completed the study may be invited to continue participating in optional follow-up visits (once at 6 months after study completion and once at the end of one year after study completion). We may ask participants to return for additional measurements, actigraphy watch use, continuous glucose monitoring, blood work, adipose tissue biopsy, and/or DEXA scan if they choose to participate in the optional follow-up.

RNA will be extracted from fat biopsy samples using standard DNA extraction kits (e.g. PureLink RNA mini kit (Life Technology)). *ADRB3, TNF,* and *IL1B* expression will be measured by isolating mRNA from adipose tissue biopsy samples and performing reverse transcription real-time PCR. ADRB3 protein levels in adipose tissue biopsy samples will be measured by Western blot. Total gene expression in subcutaneous adipose tissue samples will be analyzed using

unbiased RNA sequencing analysis. Libraries will be prepared using total RNA (e.g. TrueSeq Stranded Total RNA) and will be sequenced by Illumina (e.g. Illumina HiSeq 2500 system). Bioinformatics personnel from Dr. Saltiel's lab, in collaboration with the PI (Dr. Wilkinson), will analyze RNA-seq data to quantify gene expression using standard software. For the *ex vivo* stimulation of adipose tissue with catecholamines, in Dr. Saltiel's lab pieces of tissue will be added to media with isoproterenol, and media samples will be collected every 15 minutes for 1 hour. Supernatant will then be collected and combined with commercially available reagents which will be used to measure free glycerol and FFA production per mg of adipose tissue. Measurement of plasma biomarkers (e.g. lipids) will be performed using standard assays. All of the data collected as part of this study may be shared with Dr. Panda's lab at the Salk Institute in its de-identified form for data analysis.

myCircadianClock application ("mCC app")

The mCC app is designed to run on both Android and iOS devices that account for more than 90% of all smartphones and uses HIPAA compliant Amazon Web Server (AWS) for server-side operations. A dedicated team of developers make periodic updates to the app and to the backend server to comply with updates released by Apple, Google, and Amazon. The server side is designed to run multiple independent studies and the app is designed for individual customization. This allows study-specific customization by the investigator and user-specific customizations by participants. For this specific study, the app will be customized for time-restricted eating during the intervention period. During TRE, subjects can set their daily eating periods and receive alerts and reminders specific to TRE protocols. Subjects will receive an automated alert 15 or 30 min prior to the end of the eating interval to finish their last meal of the day. All subjects can log their food, sleep, and activity. The study coordinator can visualize real-time data from individual participants and will receive a daily summary of data logs. For food entries, the user can annotate the food picture with food name and any other descriptor (portion size, left over etc.).

Questionnaires

Questionnaires will be completed via Google Forms using a GSuite that is HIPAA compliant (BAA agreement) or on a paper form. Patients will be asked to complete the following questionnaires before and after starting the intervention period: Beck Depression Inventory (BDI), Epworth Sleepiness Scale (ESS), General Health Questionnaire Short Form-36 (SF36), Pittsburgh Sleep Quality Index (PSQI) and Munich Chronotype Questionnaire (MCTQ). They will also complete mCC app-related surveys.

Statistical analysis

Analyses for Specific Aim 1: We will run three separate linear regressions with visit 5 levels of: 1) ADRB3 mRNA and 2) protein levels and 3) *ex vivo* response of adipose tissue to catecholamines as dependent variables, respectively. For each regression we will test group differences. For each regression model, baseline levels (visit 2) of the relevant outcomes will be included as covariates. Finally, in regression models we will explore the effect of adherence, caloric intake (estimated based on the photo and/or annotation entries on the mCC app), change in physical activity (measured by wrist-worn actigraphy devices), and weight loss. **Analyses for Specific Aim 2**: Analyses will be performed as for Specific Aim 1, but for the two inflammatory markers TNFα and IL1β. **Analyses for Specific Aim 3**: Analyses will be performed as in Specific Aim 1, but with plasma FFA levels as the dependent variable. **Exploratory Aim 1:** We will examine total body changes in fat distribution and fat mass during TRE again using linear regression models. **Exploratory Aim 2:** We will examine the effects of TRE on multiple cardiometabolic biomarkers using linear regressions as described in Exploratory Aim 1. **Power calculation:** The goal sample size for enrollment is up to 120 participants. Accounting for a 15% attrition after enrollment, this will provide a sample size of 102 participants (n=51 in control, n=51 in TRE intervention). Using the independent samples t-test, this sample size (n=102) will have 80% power at the 0.05 significance level to detect an effect size of 0.7.

Randomization A study statistician will assist with randomization. We will also utilize the randomization module in RedCap as a mechanism of randomization. Participants will be randomized into the interventional TRE arm or the control group with 1:1 ratios. If multiple participants live in the same household, they will be randomized to the same group in order to maintain a consistent eating situation in the household. All investigators involved in data analysis and collection will be blinded to the randomization of participants. Only study coordinators, statistician, and dietitian will know which groups the participants are assigned to.

Banking of samples

De-identified blood samples (specifically, serum and plasma) obtained during this study will be biobanked at UCSD or the Salk Institute for possible further analyses, such as neuroendocrine, inflammatory, and/or cardiometabolic testing. There is no intention to do genetic testing. Samples will not be shared with researchers outside of those associated with this protocol. Samples will be biobanked indefinitely. We may extend the study and provide participants with the option to return for a follow-up assessment, including repeat anthropometric and laboratory measurements.

Optional Follow-Up

Upon completion of the study, subjects who are still adhering to TRE may be invited to continue participating in optional follow-up visits (once at 6 months and once at the end of one year) if subjects wish to maintain dietary intake limited to 10 hours per day and will continue to record dietary intake using the smartphone application. Subjects that were in the standard of care group may be invited to participate in follow-up visits as well and will continue to log using the application. All subjects may be asked for additional measurements, blood work, adipose tissue biopsy, and/or DEXA scan at these optional follow-up visits.

10. HUMAN SUBJECTS

We aim to enroll up to 120 participants (accounting for a 15% attrition after enrollment, this will provide a sample size of 102 participants) ≥ 18 years (all genders and races/ethnicities) with obesity (BMI ≥ 30 kg/m^2) (without T2DM) and a baseline eating duration of ≥ 14 hours/day. Participants will be recruited from the cardiovascular and internal medicine clinics at UCSD. In addition, we will utilize the bioinformatics report generated from EPIC to assist in recruitment. The inclusion and exclusion criteria are below (**Table**).

The main site of the interviews and clinical testing (vitals, blood draw, adipose tissue biopsy, DEXA) will be the UCSD Altman Clinical and Translational Research Institute (ACTRI). Blood will be processed at the UCSD Clinical Laboratory.

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Table. Inclusion and exclusion criteria

Inclusion criteria:	**Exclusion criteria:**
• Age ≥18 years • Body mass index (BMI) ≥ 30 kg/m^2 • Own a smartphone (Apple iOS or Android OS) • Baseline eating period ≥ 14 hours/day • If patients are on cardiovascular medications (HMG CoA reductase inhibitors (statins), other lipid modifying drugs (including over the counter drugs such as red yeast rice and fish oil), anti-hypertensives), no dose adjustments will be allowed during the study period	• Anti-diabetic medications or insulin within the last 6 months • Manifest diabetes, defined as fasting glucose ≥ 126 mg/dL, HbA1c ≥ 6.5%, or diagnosis of diabetes • Currently taking any medication that is meant for, or has known effect on, appetite or body weight. • Pregnant or breast-feeding • Caregiver for a dependent requiring frequent nocturnal care/sleep interruptions. Shift workers with variable (e.g. nocturnal) hours • Prolonged international travel (time zone changes) during the study period • Taking therapeutic anticoagulation which might increase risk of bleeding from fat biopsy • History of surgical intervention for weight loss • Currently enrolled in a weight-loss or weight-management program • On a special or prescribed diet for other reasons (e.g. Celiac disease) • Known inflammatory and/or rheumatologic disease • Active tobacco abuse or illicit drug use or history of treatment for alcohol abuse • History of bone marrow or solid organ transplant • History of heart failure • History of major adverse cardiovascular events (acute coronary syndrome (ACS), percutaneous coronary intervention, coronary artery bypass graft surgery, stroke/transient ischemic attack (TIA)). • History of atrial fibrillation or atrial flutter • History of malignancy, other than non-melanoma skin cancer, that is currently being treated, or that has not been treated with definitive therapy and considered to be in remission. • History of hypo- or hyperthyroidism requiring dose titration of thyroid replacement medication(s) within the past 3 months (i.e. hypothyroidism on a stable dose of thyroid replacement therapy is not an exclusion) • History of adrenal disease • History of eating disorder • History of cirrhosis • History of stage 4 or 5 chronic kidney disease or requiring dialysis • History of HIV/AIDS • Uncontrolled psychiatric disorder (including history of hospitalization for psychiatric illness). • History of obstructive sleep apnea (not on stable positive pressure therapy or other treatment for at least 3 months prior to enrollment)

11. RECRUITMENT AND PROCEDURES PREPARATORY TO RESEARCH

We will screen and recruit subjects and patients from UCSD cardiology and internal medicine clinics. We will also be screening patients through EPIC, with the help of their bioinformatics department, for research subjects who might qualify for our criteria. We will also utilize clinicaltrials.gov and ResearchMatch. We will post a flyer at UCSD facilities to promote the research study and recruit subjects. After we identify patients of interest for the study, we will contact their primary care provider or cardiologist and get their approval to potentially enroll the patient. We will ask the clinicians to provide potential participants with our contact information so they may contact the research team if they are interested in enrolling in the study. We will also ask the clinicians to specifically ask the interested patients if it is acceptable if our

research team contacts them.

We will be screening patients prior to informed consent. The following are justifications for partially waiving the HIPAA for screening purposes:

1. It allows the timely review of PHI for patients who are visiting the UCSD clinics, which will allow a greater chance of finding eligible patients. It is imperative that we consent the right patients for the study in order to:

    a. Reduce the probability of screen failures, and minimize the waste of resources, time and funds.
    b. Conduct research more smoothly and efficiently, without having to unnecessarily disturb patients who do not qualify for our study.

2. We will be using the partial HIPAA waiver only for the purposes of determining the eligibility of patients, and nothing more. This information can only be determined by screening the patients' medical records on Epic.

3. Screening cannot be practicably conducted without the use of PHI because subject eligibility depends on inclusion and exclusions factors that can only be found in their medical records. The PHI we will be using during the screening process are the patient's age, sex, past medical history, active problems, procedures, images, and medications. Only the PI and the research team will have access to this information.

4. There will be no disclosure of data to anyone outside this research group. PHI will be protected from improper use/disclosure. This will be done by securing the PHI in a locked cabinet in the PI's locked office at the Clinical Translational Research Institute (CTRI). Only the PI and the research team will have the keys to the office and the cabinet so that only they can review the documents. The PHI will never leave the office or the building.

5. The use of PHI by our research team does not involve more than minimal risk since there are no routine physical or psychological examinations or tests for screening.

6. The research could not be practicably conducted without the waiver since we have to screen the subject prior to recruiting them into the study.

7. The privacy risks are reasonable relative to the anticipated benefits of the research, since the results of the study may lead to the improvement in health of patients with obesity.

8. If the patient does not qualify, or does not agree to participate in the study, his/her PHI will not be re-used and will be destroyed at the earliest opportunity.

12. INFORMED CONSENT

See attached consent.

Informed consent will be obtained by the study coordinators or research assistants associated with this protocol. Informed consent procedures will be supervised either by the study coordinator, supervising physicians, or the principal investigator. All research personnel giving informed consent will have undergone proper training and obtained the required certificates.

The following are justifications for **partially waiving informed consent** for screening purposes:

1. It allows the timely review of PHI for patients who are visiting the UCSD clinics, which will allow a greater chance of finding eligible patients. It is imperative that we consent the right patients for the study in order to:

    a. Reduce the probability of screen failures, and minimize the waste of resources, time and funds.
    b. Conduct research more smoothly and efficiently, without having to unnecessarily disturb patients who do not

qualify for our study.

2. **Minimal Risk:** The screening procedures are considered minimal risk to the potential subjects because we will be using the partial consent waiver only for the purposes of determining the eligibility of patients, and nothing more. This information can only be determined by screening the patients' medical records on Epic.
   Screening **cannot be practicably conducted without the use of PHI** because subject eligibility depends on inclusion and exclusions factors that can only be found in their medical records. The PHI we will be using during the screening process are the patient's age, sex, past medical history, active problems, procedures, images, and medications. Only the PI and the research team will have access to this information. There will be no disclosure of data to anyone outside this research group. PHI will be protected from improper use/disclosure. This will be done by securing the PHI in a locked cabinet in the PI's locked office at the Clinical Translational Research Institute (CTRI). Only the PI and the research team will have the keys to the office and the cabinet so that only they can review the documents. The PHI will never leave the office or the building. The use of PHI by our research team also does not involve more than minimal risk since there are no routine physical or psychological examinations or tests for screening. If the patient does not qualify, or does not agree to participate in the study, his/her PHI will not be re-used and will be destroyed at the earliest opportunity.

3. **Rights and Welfare of Subjects:** The waiver of consent would not adversely affect the rights and welfare of the potential subjects. Their standard of care in the hospital will remain the same regardless of the partial waiver of consent.

4. The potential subject will be informed about the purpose of the study, the duration of the study, the activities they will be doing in the study, the risks and benefits, expenses, compensation, alternatives to participating, privacy, their rights as research subjects and study contact information.

5. The privacy risks are reasonable relative to the anticipated benefits of the research, since the results of the study may lead to the improvement in health of patients with obesity.

13. ALTERNATIVES TO STUDY PARTICIPATION

Subjects have the right to refuse participation in the study. Patients will have no changes to their current medical regimen. They can choose to stop participating at any time.

14. POTENTIAL RISKS

1. Questionnaires/surveys: Some questions may make the participant uncomfortable. Participant may decline to answer survey questions or participate in the app's tasks. If a survey question makes them feel uncomfortable, they may leave questions blank.
2. Randomization: Given that subjects will be randomized to one of two groups, those in the control group will not experience the potential benefits of the intervention group (TRE).
3. Smartphone use: As with any smartphone application, prevailing laws should be followed about when and where to use the smartphone. Follow local and federal regulations about the usage of smartphone in specific areas. Additionally, the app should not be used in any capacity to perform or document illegal activity. The Salk Institute for Biological Studies, Dr. Satchidananda Panda and all members of his research team, including collaborators, are not liable for any illegal activity that is performed, captured, or stored by the myCircadianClock app. Participation in this study does not require the participant to change anything related to the smartphone account or data plan. The app can use either an existing mobile data plan or WiFi connections. The app can be configured to use only WiFi if participant wishes to limit the impact on data usage.
4. Venipuncture: pain, a bruise at the point where the blood is taken, discoloration, redness and swelling of the vein and infection.
5. Wrist-worn actigraphy device: Participants will be asked to wear a wrist-worn actigraphy device. Despite all the measures taken in material selection, design and manufacturing to ensure biocompatibility, some people may not tolerate wearing the device and can develop skin irritations. If this happens, participants will be asked to notify the study personnel immediately. The instructions for use of the watch advise on the measures participants can take to

limit the chance of experiencing any problems (such as tightness of wearing and regular cleaning).

6. Use of continuous glucose monitor: redness at the sensor insertion site, skin irritation (erythema/edema), local infection, inflammation, pain or discomfort, bleeding at the glucose sensor insertion site, bruising, itching, scarring or skin discoloration, hematoma, tape irritation, and sensor or needle fracture during insertion, wear or removal.
7. Adipose tissue biopsy: adverse reaction (such as allergy, temporary numbness, tingling sensations) to the local anesthetic used (lidocaine); pain, bruising, infection or bleeding at the site of biopsy; discomfort (including allergy to tape and dressings) related to the dressing applied to the biopsy site. It is possible that the biopsy site will scar. There is a very small chance that the scars from the biopsy sites may become thick (hypertrophy).
8. Dual energy x-ray absorptiometry (DEXA) scan: As with any form of radiation, there is a carcinogenic or genetic risk. However, the risks are assumed to be minimal as this is a low-energy X-ray modality that uses a low dose to image the body. During participation in this research study, subject will be exposed to radiation from scheduled DEXA scans. The total exposure resulting from these imaging studies is calculated to be approximately 0.09000mSv. This amount is less than one would receive from one year of natural exposure in the San Diego area, which is approximately 1.6 mSv. Cumulative exposure from radiation may increase risk of developing certain types of cancer in the future. The principal investigator for this research study has determined and verified that some of the x-rays and/or imaging scans prescribed for this study would typically be performed as part of the standard medical care required to adequately monitor the current illness. If subject is especially concerned with radiation exposure, or has had a lot of x-rays or imaging scans already, subject will be advised to discuss this with the principal investigator for this study or their regular doctor.
9. Reduced daily feeding period (from ≥ 14 hours per day to 10 hours per day): hunger, fatigue, hypoglycemia
10. Fasting (for at least 12 hours prior to study visit) for adipose tissue biopsies and venipuncture: hunger, fatigue, and hypoglycemia.
11. Inability to start lipid-lowering, anti-hypertensive, and anti-diabetics medications or undergo dose adjustments of these medications during the study period: potential risk of incompletely controlled lipid, blood pressure, and/or blood sugar values during the study period which could have either acute (e.g. symptomatic high or low blood pressure, symptomatic high or low blood sugars) and/or long-term risks (e.g. potential contribution to long-term risk of cardiovascular events). However, if any of the monitored parameters during this study become abnormal to the point of necessitating immediate treatment based on the judgement of the investigators or participant's primary physician or cardiologist, the participant will be advised to immediately stop the study and seek medical attention.
12. Unscheduled visits: There may be the possibility of having subjects return for unscheduled visits in between the scheduled visits. This would be for the following reasons: PI discretion, if the bloodwork needs to be redrawn due to lab or processing errors, repeat adipose tissue biopsy because of inadequate or uninterpretable sample, and/or reapplication of the continuous glucose monitor and/or actigraphy watch if it fell off or did not record data. Participants will not be given additional compensation for unscheduled visits.
13. A potential loss of confidentiality: The UCSD Institutional Review Board (IRB), the FDA, and other government agencies may inspect the study records. To minimize the risk of a potential loss of confidentiality, subjects will be assigned a unique subject code and their blood samples will be assigned a different unique specimen code. A separate paper document will link patient identifiers with subject codes and specimen codes. This document will be kept in a secure location in a locked cabinet. Only the PI and study coordinator will have the key to the cabinet. No research documents will have any patient identifiers and will only be labeled with the unique assigned subject codes.

15. RISK MANAGEMENT PROCEDURES AND ADEQUACY OF RESOURCES

Strict sterile technique will be observed during venipuncture. The risks of taking blood include pain, a bruise at the point where the blood is taken, discoloration, redness and swelling of the vein and infection. Pressure will be held at the site of venipuncture to minimize bruising, blood loss and swelling.

Strict sterile technique will also be observed during adipose tissue biopsy. The risks of the adipose tissue biopsy include pain, bruising and/or bleeding at the biopsy site, discoloration, redness, swelling and infection. Pressure will be held at the site of the adipose tissue biopsy to minimize bruising, blood loss, and swelling.

All subjects are screened with a medical history and physical exam to ensure none has health problems that put them at risk. To minimize bleeding complications, coagulation parameters must be normal for participation and patients will be

excluded if they are taking therapeutic anticoagulation. Blood drawing will be minimized to no greater than 200 ml per patient. Subjects will be thoroughly informed as to these potential problems.

A psychiatric assessment conducted by a psychiatrist or clinical psychologist will be offered if any of the following events occurs: 1) request from a subject, a subject's family member, or research staff; 2) An increase on item #9 of BDI-II which assesses suicidal ideation or if suicidal intentions are brought to the attention of the study staff; 3) any increase from baseline of >20% on the BDI-II, 4) answering "yes" to the question "do you feel that your mood symptoms have worsened" during the clinic visit check-ins. BDI-II data will be reviewed within 24 hours of each assessment during the study. At any time point, patients found to have suicidal ideation, as assessed by BDI-II question #9 scoring >0, will be further assessed by the comprehensive suicide risk assessment tool (http://www.healthquality.va.gov/guidelines/MH/srb/VADODCP_SuicideRisk_Full.pdf) and/or follow-up with a health professional. Thus, we will assess suicidal symptoms in a way that is consistent with published clinical guidelines from major medical institutions. Those found to be at moderate or high suicide risk at the baseline assessment will not be included in the study and those who develop this level of risk during the study will be removed from the study. In all cases, participants will be connected to mental health providers immediately at a level of care appropriate to the suicide risk. This may include phoning, with their consent, their mental health provider for treatment planning or escorting them to the ER or Mental Health Access Clinic for urgent/emergent care.

Because this is a low risk study, a Data Safety Monitoring Board (DSMB) is not necessary. Instead, a Data Safety Monitoring Plan (DSMP) is provided.

A DSMB is not needed for this study for various reasons:

1. This is a low risk study assessing the effect of reducing the total number of hours in a day a participant may consume food and beverage.
2. The study does not involve a high-risk intervention or a vulnerable population, so DSMB should not be needed.
3. This is a single site study that is **not intended** to evaluate treatments intended to prolong life or reduce risk of a major adverse health outcome.
4. This study is **not intended** to compare rates of mortality or major morbidity.
5. This study is addressing lesser outcomes, such as promoting weight loss.
6. The study population is not at an elevated risk of more severe outcomes.

DATA SAFETY MONITORING PLAN (DSMP)

Oversight responsibilities

Oversight of the trial is provided by the Principal Investigator (PI), Dr. Wilkinson.

Monitoring procedures

Dr. Wilkinson assures that informed consent is obtained prior to performing any research procedures, that all subjects meet eligibility criteria, and that the study is conducted according to the IRB-approved research plan.
Study data are accessible at all times for the PI to review. The PI reviews study conduct such as accrual, drop-outs, protocol deviations on a monthly basis. The PI reviews AEs individually real-time and in aggregate on a weekly basis. The PI reviews serious adverse events (SAEs) in real-time. The PI ensures all protocol deviations, AEs, and SAEs are reported to the IRB according to the applicable regulatory requirements.

Collection and reporting of SAEs and AEs

For this study, the following standard AE definitions are used:

Adverse event: Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure, regardless of whether it is considered related to the medical treatment or procedure.

Serious Adverse Event: Any AE that results in any of the following outcomes:

7. Death

8. Life-threatening

9. Event requiring inpatient hospitalization or prolongation of existing hospitalization

10. Persistent or significant disability/incapacity

AEs are graded according to the following scale:

Mild: An experience that is transient and requires no special treatment or intervention. The experience does not generally interfere with usual daily activities. This includes transient laboratory test alterations.
Moderate: An experience that is alleviated with simple therapeutic treatments. The experience impacts usual daily activities. Includes laboratory test alterations indicating injury, but without long-term risk.
Severe: An experience that requires therapeutic intervention. The experience interrupts usual daily activities. If hospitalization (or prolongation of hospitalization) is required for treatment it becomes an SAE.

The study uses the following AE attribution scale:

Not related: The AE is clearly not related to the study procedures (i.e., another cause of the event is most plausible and/or a clinically plausible temporal sequence is inconsistent with the onset of the event).
Possibly related: An event that follows a reasonable temporal sequence from the initiation of study procedures, but that could readily have been produced by a number of other factors.
Related: The AE is clearly related to the study procedures.

AEs are identified immediately after the study procedure. We will have the patient rest for 30 minutes to an hour after the procedure or activity to observe him or her for any AEs. We will follow up with the patient the next day and once a week for the following weeks leading up to their next appointment with us. Dr. Wilkinson who is a cardiologist and a team of nurses will always be nearby the patient at the appointment in case an event occurs.

SAEs and specific procedure-associated AEs are reported to the PI and IRB within 24 hours. In addition, all AEs are reported according to the IRB AE reporting guidelines.

Management of risks to subjects

Expected AEs

1. Blood draw
    a. Hematoma
    b. Arterial puncture
    c. Pain
    d. Nerve damage
    e. Re-bleeding
    f. Allergy
    g. Phlebitis
    h. Vasovagal reaction
    i. Anxiety/fear
11. Wrist-worn actigraphy device:
    a. Skin irritation.
12. Use of continuous glucose monitor
    a. Redness at the sensor insertion site
    b. Skin irritation (erythema/edema)
    c. Local infection, inflammation, pain or discomfort
    d. Bleeding at the glucose sensor insertion site
    e. Bruising, itching, scarring or skin discoloration, hematoma, tape irritation, and sensor or needle fracture during insertion, wear or removal.

13. Reduced daily feeding period (from ≥ 14 hours per day to 10 hours per day)
    a. Hunger
    b. Fatigue
    c. Hypoglycemia
14. Inability to start lipid-lowering, anti-hypertensive, and anti-diabetics medications or undergo dose adjustments of these medications during the study period
    a. Incompletely controlled lipid, blood pressure, and/or blood sugar values during the study period which could have either acute (e.g. symptomatic high or low blood pressure, symptomatic high or low blood sugars) and/or long-term risks (e.g. potential contribution to overall long-term risk of cardiovascular disease/events).
15. Adipose tissue biopsy:
    a. Hematoma
    b. Pain
    c. Nerve damage
    d. Bleeding
    e. Allergy
    f. Vasovagal reaction
    g. Anxiety/fear
16. DEXA:
    a. Low dose radiation

AE Management

1. Blood Draw:
    a. Prior to enrollment into the study, the patient will be educated on the risks of the blood draw. The phlebotomist will calm the patient beforehand to reduce anxiety/fear and minimize the probability of vasovagal response. Only sterile needles and gauze will be used to prevent allergic reactions and infection. The area of the blood draw will be thoroughly sanitized with an alcohol wipe prior to the needle stick. Care will be used when penetrating the skin with the needle so that there is a minimized risk of punctured artery and damaged nerve. The needle stick will be done swiftly and securely by an experienced phlebotomist in order to reduce pain. Pressure will be applied on the area immediately after the needle stick to minimize the risk of hematoma. The phlebotomist will inform the patient on when and how to undress their puncture site. Dr. Wilkinson, who is a cardiologist, will be overseeing the procedure to check for the patient's vital signs. Dr. Wilkinson's phone number will be given to the patient for any questions or concerns that may arise in the future.

    b. In the event of an AE that occurs during the appointment, the facility (CTRI) is equipped with trained medical staff (physicians and nurses) who can respond quickly. Should an event occur, Dr. Wilkinson and the nurses will be nearby to administer immediate medical care and send the patient to the nearest hospital. If the AE occurs after the appointment, the patient will be referred for prompt medical attention. The patient will be withdrawn from the study. Dr. Wilkinson will monitor the patient until the problem has been resolved or has stabilized.

2. Actigraphy device:
    a. If participants develop skin irritation, they will be asked to notify the study personnel immediately.
    b. The instructions for use of the watch advise on the measures participants can take to limit the chance of experiencing any problems (such as tightness of wearing and regular cleaning).

3. Use of continuous glucose monitor:

    a. Prior to enrollment into the study, the patient will be educated on the risks of wearing a continuous glucose

monitor. To minimize risk, the continuous glucose monitor will be placed and removed only during study visits by a trained member of the study team. The device will be inserted using the following protocol: 1) Wash hands with soap and water, 2) Choose sensor insertion site, 3) Clean the skin over the insertion site with an alcohol wipe, 4) A skin adhesive product may or may not be used, 5) The sensor delivery device will then be used to position the sensor under the participant's skin. In the event of bleeding at the insertion site, the patient will be advised to hold pressure over the area and present for medical evaluation. For other local site issues, such as irritation, discomfort, or redness, if these symptoms are unacceptable to the participant the study team will immediately assist the participant with removal of the device and the patient will be removed from the study and will be monitored for resolution of symptoms. Sensor or needle fracture will be addressed immediately by the study investigators with assistance from the device manufacturer.

b. In the event of an AE that occurs during the appointment, the facility (CTRI) is equipped with trained medical staff (physicians and nurses) who can respond quickly. Should an event occur, Dr. Wilkinson and the nurses will be nearby to administer immediate medical care and send the patient to the nearest hospital. If the AE occurs after the appointment, the patient will be referred for prompt medical attention. The patient will be withdrawn from the study. Dr. Wilkinson will monitor the patient until the problem has been resolved or has stabilized.

4. Reduced daily feeding period (from ≥ 14 hours per day to 10 hours per day)

   a. Prior to enrollment into the study, the patient will be educated on the risks of reducing the total number of hours of oral intake during a 24 hour period. The participant will be advised that hunger and/or subjective feelings of fatigue or malaise may occur. Risk of hypoglycemia will be discussed, especially in those patients with impaired fasting glucose. Participants should immediately consume sugar (e.g. candies, glucose) in the event of symptomatic hypoglycemia.

   b. In the event of an AE, the patient will be referred for prompt medical attention and may be withdrawn from the study. Dr. Wilkinson will monitor the patient until the problem has been resolved or has stabilized.

5. Inability to start lipid-lowering, anti-hypertensive, and anti-diabetics medications or undergo dose adjustments of these medications during the study period
   a. Because initiation or adjustment of lipid-lowering, anti-hypertensive, and anti-diabetes medications will confound the results of this study, these medication changes cannot occur during the study period without a participant being withdrawn from the study. Prior to enrollment, the participant will be educated on the potential risks associated with incompletely controlled lipid, blood pressure, and/or blood sugar values during the study period, including symptomatic high or low blood pressures and symptomatic high or low blood sugars. Prior to enrollment, the participant will also be educated regarding the potential contribution to long-term risk of cardiovascular disease/events associated with a 12 week study period during which medication adjustments are not allowed, however it will be noted that such long-term risks associated with this aspect of the study cannot be quantified.
   b. In the event of an AE, the patient will be referred for prompt medical attention and will be withdrawn from the study so that medication changes can be made. Dr. Wilkinson will monitor the patient until the problem has been resolved or has stabilized.

6. Adipose tissue biopsy:
   a. Prior to enrollment into the study, the patient will be educated on the risks of the adipose tissue biopsy. Research staff will calm the patient beforehand to reduce anxiety/fear and minimize the probability of vasovagal response. Only sterile needles and gauze will be used to prevent allergic reactions and infection. The area of the biopsy will be thoroughly sanitized with an alcohol wipe prior to the needle stick. Care will be used when penetrating the

skin with the needle so that there is a minimized risk of punctured artery and damaged nerve. The needle stick will be done swiftly and securely by an experienced provider in order to reduce pain. Pressure will be applied on the area immediately after the needle stick to minimize the risk of bleeding and hematoma. The provider performing the biopsy will inform the patient on when and how to undress their puncture site. Dr. Wilkinson, who is a cardiologist, will be performing or overseeing the procedure. Dr. Wilkinson's phone number will be given to the patient for any questions or concerns that may arise in the future.

b. In the event of an AE that occurs during the appointment, the facility (CTRI) is equipped with trained medical staff (physicians and nurses) who can respond quickly. Should an event occur, Dr. Wilkinson and the nurses will be nearby to administer immediate medical care and send the patient to the nearest hospital. If the AE occurs after the appointment, the patient will be referred for prompt medical attention. The patient will be withdrawn from the study. Dr. Wilkinson will monitor the patient until the problem has been resolved or has stabilized.

7. DEXA:
   a. Prior to enrollment into the study, the patient will be educated on the risks of obtaining a DEXA scan. To minimize risks, standard protocol will be followed. In the case that the patient is no longer comfortable or changes his/her mind, the DEXA scan will be stopped.
   b. In the event of an AE that occurs during the appointment, the facility (ACTRI) is equipped with trained medical staff (physicians and nurses) who can respond quickly. Should an event occur, Dr. Wilkinson and the nurses will be nearby to administer immediate medical care and send the patient to the nearest hospital. If the AE occurs after the appointment, the patient will be referred for prompt medical attention. The patient will be withdrawn from the study. Dr. Wilkinson will monitor the patient until the problem has been resolved or has stabilized.

In the event that a patient either withdraws from the study or the investigator decides to discontinue a patient due to SAE, the patient will have appropriate follow-up medical monitoring. Monitoring will continue until the problem requiring hospitalization has resolved or stabilized with no further change expected, is clearly unrelated to study medication, or results in death.

Plan for data management

Data will be collected using standardized paper forms and will only be identified with the study's ID of the participant. The codes that link the name of the participant and the study ID will be kept confidential by the Principal Investigator in a secured cabinet. Data will be entered in the computer independently by UCSD certificated and trained data entry staff, and discrepancies corrected by a supervisor based on source documents.

mCC app: To avoid losing or publicly exposing any collected data on the server, data will be backed up in a separate back-up cloud server. This system uses multiply redundant hardware and software strategies to protect against data loss. By design, the filenames include a randomly generated string, date and time in the filename making it virtually impossible to guess a particular filename. We use the geolocation data for additional utility will be used to evaluate the individual's daily eating, activity pattern associated with access to healthy food, public park, etc. In addition, we shall specifically advise the subject regarding limits imposed by certain wireless carriers (e.g. AT&T) on data uploads, as well as the fact that depending on their device settings the location at which a food photo is clicked may be included in the EXIF information for the JPEG file transmitted to our servers.

Data quality will be monitored by random inspection of the completed forms by the PI. If necessary, re-training of data collectors will be conducted.

16. PRIVACY AND CONFIDENTIALITY CONSIDERATIONS INCLUDING DATA ACCESS AND MANAGEMENT

All study forms including consents, HIPAAs, and case report forms (CRFs), which will include elements from the present and past history, patient demographics, including age, current medications and lab draws, will be stored in in a locked cabinet in Dr. Wilkinson's office in the ACTRI building. Only approved study personnel will have access to this

information. To minimize the potential loss of confidentiality, patients will be assigned a unique number as their subject identifier code. The unique subject code will be used to label all study documents.

Some portions of the specimens collected may be sent to a central laboratory outside of UCSD for testing. All samples will be labeled with a unique specimen code and the only way to link the specimens with the subject code and any personal health identifiers will be on a physical sheet of paper locked in a cabinet in Dr. Wilkinson's office in the ACTRI building.

Social security numbers will be collected only for purposes of subject payment. This information will only be available to study personnel and will be secured in the same manner mentioned above.

17. POTENTIAL BENEFITS

Subjects may or may not benefit directly from participating in the study. The potential benefits to society in general include improved understanding of the effects of time-restricted eating on the health of individuals with obesity.

18. RISK/BENEFIT RATIO

We believe that risks are minimal in this study and are outweighed by the potential cardiometabolic benefits of time-restricted eating in people with obesity, including weight loss.

19. EXPENSE TO PARTICIPANT

There will be no expense to participants.

20. COMPENSATION FOR PARTICIPATION

Patients will be compensated $300 for fully participating in the study. Parking will be provided for. $25 will also be dispensed at optional follow-up visits (once at 6 months and once at the end of one year) if participants choose to return for these visits.
Participants will receive the following compensation per visit:
Visit 1: $25
Visit 2: $75
Visit 3: $75
Visit 4: $25
Visit 5: $100

21. PRIVILEGES/CERTIFICATIONS/LICENSES AND RESEARCH TEAM RESPONSIBILITIES

Michael Wilkinson, MD (Principal Investigator) is an Assistant Professor of Medicine at UCSD and is board certified in internal medicine and cardiology. He has clinical privileges at UCSD Medical Center. He will be responsible for all aspects of the project including project design, oversight of project and study personnel, patient recruitment, IRB submissions, data analysis, and manuscript writing.

Alan Saltiel, PhD (Co-Investigator) is a Professor at UCSD and is responsible for project oversight and will oversee studies related to analyses of adipose tissue biology in biopsied samples. He has extensive expertise in the studies of adipose tissue metabolism proposed in this study.

Pam Taub, MD (Co-Investigator) is an Associate Professor of Cardiology and experienced clinical and translational researcher at UCSD who has abundant experience in clinical research, including metabolic research examining the effects of time-restricted eating (TRE) as a dietary intervention to treat metabolic diseases in collaboration with Dr. Wilkinson. She will provide insight into clinical trial study design and execution and assist with interpretation of study findings.

Satchidananda Panda, PhD (Co-Investigator) is a Professor at the Salk Institute and UCSD. He is an expert in circadian rhythm biology and TRE, and has performed translational research laying the foundation for the study proposed in this application. This includes studying the effects of TRE in individuals with metabolic syndrome in collaboration with Dr. Wilkinson. He will provide insight into study design and findings.

Joseph Witztum, MD (Co-Investigator) is a UCSD Distinguished Professor of Medicine and a world-expert in lipid metabolism. He will provide insight into study findings.

Emily Manoogian, PhD (Co-Investigator) is a researcher (post-doc) at the Salk Institute for Biological Studies, in the lab of Dr. Satchidananda Panda. She will be responsible for the following aspects of the project: project design, data analysis, manuscript writing, and providing mCC app support.

Shannon Reilly, PhD (Co-Investigator) is an Assistant Adjunct Professor of Medicine at UCSD. She has extensive experience performing adipose tissue biopsies and performing the analyses of adipose tissue metabolism proposed in this study. She has collaborated extensively with Dr. Alan Saltiel. She will provide consultation on performing adipose tissue biopsies, as well as performing and interpreting the results of the experiments of adipose tissue metabolism.

Ashley Rosander (Research Coordinator) has undergone proper training and obtained the certificates necessary to work with human subjects and she will manage and assist with the following: patient recruitment, patient scheduling, IRB submissions, administrative and technical support, data collection and analysis.

Aryana Pazargadi (Research Coordinator) has undergone proper training and obtained the certificates necessary to work with human subjects. She will assist with the following: patient recruitment, patient scheduling, IRB submissions, administrative and technical support, and data collection.

Hannah Lo (Research Coordinator) has undergone proper training and obtained the certificates necessary to work with human subjects. She will assist with the following: patient recruitment, patient scheduling, IRB submissions, administrative and technical support, and data collection.

Erika Padilla (Research Coordinator) has undergone proper training and obtained the certificates necessary to work with human subjects and she will manage and assist with the following: patient recruitment, patient scheduling, IRB submissions, administrative and technical support, data collection and analysis.

David Van (Research Coordinator) has undergone proper training and obtained the certificates necessary to work with human subjects and he will manage and assist with the following: patient recruitment, patient scheduling, IRB submissions, administrative and technical support, and data collection.

Victoria Green (Research Coordinator) has undergone proper training and obtained the certificates necessary to work with human subjects and she will manage and assist with the following: patient recruitment, patient scheduling, IRB submissions, administrative and technical support, and data collection.

Justina Nguyen (Research Coordinator) has undergone proper training and obtained the certificates necessary to work with human subjects and she will manage and assist with the following: patient recruitment, patient scheduling, IRB submissions, administrative and technical support, and data collection.

Juancarlos Cancilla (Research Coordinator) has undergone proper training and obtained the certificates necessary to work with human subjects and he will manage and assist with the following: patient recruitment, patient scheduling, IRB submissions, administrative and technical support, and data collection.

Shelley Mitra (Student Research Volunteer) has undergone proper training and obtained the certificates necessary to work with human subjects and she will manage and assist with the following: patient recruitment, patient scheduling, IRB submissions, administrative and technical support, and data collection.

Sneha Panda (Student Research Volunteer) has undergone proper training and obtained the certificates necessary to work with human subjects and she will manage and assist with the following: patient recruitment, patient scheduling, IRB submissions, administrative and technical support, and data collection.

Nikko Gutierrez (Research Assistant) has undergone proper training and obtained the certificates necessary to work with human subjects and he will manage and assist with the following: administrative and technical support, data collection and analysis, mCC app support. Nikko is also a research assistant in Dr. Panda's lab at the Salk Institute.

Kyla Laing (Research Assistant) has undergone proper training and obtained the certificates necessary to work with human subjects and she will manage and assist with the following: administrative and technical support, data collection and analysis, mCC app support. Kyla is also a volunteer in Dr. Panda's lab at the Salk Institute.

Banyamin Dadpey (Research Assistant) has undergone proper training and obtained the certificates necessary to work with human subjects and he will manage and assist with procedures and analyses related to the adipose tissue samples.

Julia H. Deluca (Research Assistant) has undergone proper training and obtained the certificates necessary to work with human subjects and she will manage and assist with procedures and analyses related to the adipose tissue samples.

All blood draws will be performed by research personnel trained in phlebotomy.

22. BIBLIOGRAPHY

1. Wilkinson MJ, Manoogian ENC, Zadourian A, et al. Ten-Hour Time-Restricted Eating Reduces Weight, Blood Pressure, and Atherogenic Lipids in Patients with Metabolic Syndrome. Cell Metab 2020;31:92-104 e5.
2. Chaix A, Zarrinpar A, Miu P, Panda S. Time-restricted feeding is a preventative and therapeutic intervention against diverse nutritional challenges. Cell Metab 2014;20:991-1005.
3. Benjamin EJ, Muntner P, Alonso A, et al. Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. Circulation 2019;139:e56-e528.
4. Kotsis V, Jordan J, Micic D, et al. Obesity and cardiovascular risk: a call for action from the European Society of Hypertension Working Group of Obesity, Diabetes and the High-risk Patient and European Association for the Study of Obesity: part A: mechanisms of obesity induced hypertension, diabetes and dyslipidemia and practice guidelines for treatment. J Hypertens 2018;36:1427-40.
5. Carmen GY, Victor SM. Signalling mechanisms regulating lipolysis. Cell Signal 2006;18:401-8.
6. Robidoux J, Cao W, Quan H, et al. Selective activation of mitogen-activated protein (MAP) kinase kinase 3 and p38alpha MAP kinase is essential for cyclic AMP-dependent UCP1 expression in adipocytes. Molecular and cellular biology 2005;25:5466-79.
7. Mowers J, Uhm M, Reilly SM, et al. Inflammation produces catecholamine resistance in obesity via activation of PDE3B by the protein kinases IKKepsilon and TBK1. Elife 2013;2:e01119.
8. Makino H, Saijo T, Ashida Y, Kuriki H, Maki Y. Mechanism of action of an antiallergic agent, amlexanox (AA-673), in inhibiting histamine release from mast cells. Acceleration of cAMP generation and inhibition of phosphodiesterase. Int Arch Allergy Appl Immunol 1987;82:66-71.
9. Oral EA, Reilly SM, Gomez AV, et al. Inhibition of IKKvarepsilon and TBK1 Improves Glucose Control in a Subset of Patients with Type 2 Diabetes. Cell Metab 2017;26:157-70 e7.
10. Gill S, Le HD, Melkani GC, Panda S. Time-restricted feeding attenuates age-related cardiac decline in Drosophila. Science 2015;347:1265-9.
11. Hatori M, Vollmers C, Zarrinpar A, et al. Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. Cell Metab 2012;15:848-60.

23. FUNDING SUPPORT FOR THIS STUDY

1. UCSD/UCLA Diabetes Research Center (DRC) Pilot and Feasibility Grant (PI: Michael Wilkinson, MD)

24. BIOLOGICAL MATERIALS TRANSFER AGREEMENT

MTA with Salk is fully executed as of 10/29/20.

25. INVESTIGATIONAL DRUG FACT SHEET AND IND/IDE HOLDER

None

26. IMPACT ON STAFF

None

27. CONFLICT OF INTEREST

The study investigators and staff listed under section 21 above have no conflicts of interest to disclose.

28. SUPPLEMENTAL INSTRUCTIONS FOR CANCER-RELATED STUDIES

None

29. OTHER APPROVALS/REGULATED MATERIALS

None

30. PROCEDURES FOR SURROGATE CONSENT AND/OR DECISIONAL CAPACITY ASSESSMENT

Only patients who are competent to provide consent will be enrolled in the study.