4 December 2024

Study Protocol and Statistical Analysis Plan*

Manoogian ENC, Wilkinson MJ, O'Neal M, et al. Time-restricted eating in adults with metabolic syndrome. A randomized controlled trial. Ann Intern Med. 1 October 2024. [Epub ahead of print]. doi:10.7326/M24-0859

Protocol for:
Time-Restricted Eating in Adults with Metabolic Syndrome:
A Randomized Control Trial
NCT04057339

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Note: There was a typo in the inclusion/exclusion section of the protocol that listed systolic blood pressure levels of 135 mmHg instead of 130 mmHg to qualify as elevated blood pressure as a factor of metabolic syndrome. It was noted correctly as 130 mmHg in the recruitment section of the protocol and was used for all recruitment and screening.

1. Final Protocol

UCSD Human Research Protections Program
New Biomedical Application
RESEARCH PLAN

Instructions for completing the Research Plan are available on the HRPP website.
The headings on this set of instructions correspond to the headings of the Research Plan.
General Instructions: Enter a response for all topic headings.
Enter "Not Applicable" rather than leaving an item blank if the item does not apply to this project.
Version date: 10/26/2021

1. PROJECT TITLE

Influence of time-restricted eating (TRE) on circadian regulation of glucose homeostasis and mitochondrial function—The TIMET Study

2. PRINCIPAL INVESTIGATOR

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3. FACILITIES

UCSD Hospitals and Clinics
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Institute for Biological Studies

4. ESTIMATED DURATION OF THE STUDY

5 years

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

Circadian rhythms optimize nutrient homeostasis by orchestrating catabolic and anabolic metabolism to appropriate times of the 24 hour (h) day. Chronic circadian rhythm disruption predisposes individuals to metabolic diseases including obesity and type 2 diabetes. Conversely, maintaining a daily rhythm of feeding and fasting cycles sustains a robust circadian rhythm which improves cellular bioenergetics and results in improved metabolism. Time-restricted eating (TRE) is a specific feeding-fasting pattern in which feeding is restricted to 8-12 h a day.

In a randomized controlled trial, we intend to measure the health impact of TRE in patients with metabolic syndrome (with three or more of the following criteria: increased waist circumference, abnormal cholesterol levels, elevated blood pressure, or elevated blood sugar), who habitually eat over a period of 12 or more hours . 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 an eating window of 8- 10 hours (a minimum of 4 hours less than their baselines window) for 12 weeks (TRE). We will evaluate the impact of TRE on blood glucose levels, metabolic biomarkers, body composition, and mitochondrial function. These assessments will be made at baseline and at the end of the 12-week intervention period. Food/drink intake, activity, and sleep will be monitored with the smartphone myCircadianClock application ("mCC app") throughout the study. There is also an optional additional imaging-based assessment of liver fat (magnetic resonance imaging derived proton density fat fraction (MRI-PDFF)) for patients who meet study criteria and are also diagnosed with non-alcoholic fatty liver disease.

6. SPECIFIC AIMS

We hypothesize that imposing consistent feeding-fasting cycles will restore the equilibrium between catabolic and anabolic processes, which will improve glucose homeostasis and cardiometabolic biomarkers, decrease abdominal fat and enhance mitochondrial function. We will test this hypothesis with the following specific aims:

Specific Aim 1 – Evaluate the impact of TRE on glucose homeostasis.

Hypothesis 1: The TRE group will have a significant decrease in HbA1c levels compared to the control group at the end of the study. The primary endpoint will be the change in glucose levels assessed via HbA1c.

Sub-aim 1A: Assess changes in other glycemic measures including fasted blood glucose, insulin, HOMA-IR and continuous glucose monitors (CGM).
Hypothesis: The TRE group will have a significant improvement in glycemic parameters (assessed via fasting glucose, HOMA-IR, Insulin, and CGMs) compared to the control group and compared to baseline.

Specific Aim 2 – Assess changes in cardiometabolic biomarkers in response to TRE.
Hypothesis 2: The TRE group will significantly improve cardiometabolic biomarkers compared to the control group.

Specific Aim 3 – Assess the impact of TRE on body composition.
Hypothesis 3: The TRE group will have significantly lower abdominal fat mass compared to the control group at the end of the study as assessed by DXA.

Exploratory Aim 1 – Elucidate the effects of TRE on changes in mitochondrial structure and gene expression in skeletal muscle.
Impaired glucose homeostasis can result from insulin resistance, compromised mitochondrial function, adiposity, dyslipidemia, and inflammation. To elucidate the underlying mechanisms, skeletal muscle biopsies will be utilized to analyze 1) alterations in mitochondrial structure/function and 2) changes in gene expression of candidate target genes triggered by TRE.

Exploratory Aim 2 – Evaluate for change in hepatic steatosis (liver fat), quantified using magnetic resonance imaging derived proton density fat fraction (MRI-PDFF), after 12-weeks of TRE.
High levels of hepatic fat are closely associated with cardiovascular disease risk markers such as obesity, insulin resistance and metabolic syndrome. Reduced liver fat could serve as an imaging marker that heralds reduction in cardiovascular and liver disease risk with TRE in this population. To assess whether there is a reduction from baseline intra-hepatic lipid content with TRE, MRI-PDFF scans will be utilized.

7. BACKGROUND AND SIGNIFICANCE

Metabolic syndrome. In the United States, >34% of adults meet criteria for metabolic syndrome, and prevalence increases with age. Metabolic syndrome is characterized by the presence of multiple risk factors for cardiovascular disease and diabetes (see criteria outlined in Table 1) [1, 2]. Compared to unaffected individuals, the presence of metabolic syndrome doubles the risk of developing cardiovascular disease over the next 5 to 10 years, and it is associated with a 5-fold increase in the risk of type-II diabetes mellitus (T2DM) [1]. In patients with T2DM, cardiovascular disease is the leading cause of death.

While lifestyle modification is currently the first line of treatment for metabolic syndrome, it is usually not very effective as the patients' condition often deteriorates. The diet currently recommended for patients with metabolic disease is not ideal [3]. Second, lifestyle programs administered in a clinical setting rely heavily on weight loss through calorie-reduced diets, which is an approach that is effective at reducing body weight and improving cardio-metabolic risk factors in the short- to medium-term, but often fails in the long-term given that many individuals fail to adhere to low-calorie diets for more than 6 months [4]. Moreover, for patients with clinically normal body weight and metabolic syndrome, there is very little room for weight loss. Thus, there is an unmet clinical need to identify better approaches for long-term body weight control and lifestyle modifications that affect glucose homeostasis-independent of changes in adiposity. *Therefore, novel mechanisms that can act independently of weight loss or altered nutritional content are needed.*

Metabolic diseases: Disruption in metabolic homeostasis underlies metabolic diseases. Nutrient homeostasis (e.g. of glucose, lipid, cholesterol and amino acid metabolism) is achieved by concerted action of (a) sensors that detect the cellular or organismal nutrient demand and nutrient availability, which in turn instruct the regulators and enzymes that mediate (b) anabolic or (c) catabolic pathways [5-7]. Several intermediate metabolites serve as co-factors, ligands, scavengers, electron/proton/carbon/active group carriers (e.g. Heme, sterols, bile acids, nicotinamide adenine dinucleotide (NAD+)) for anabolic or catabolic reactions. Research on metabolic homeostasis has led to the identification of these sensors, regulators, enzymes, and intermediate metabolites which upon hyper- or hypo-activation disrupts homeostasis thus leading to metabolic diseases. Overall, homeostasis is maintained by equilibrium between anabolic and catabolic pathways. *One mechanism contributing to this equilibrium is the circadian rhythm, such that anabolic and catabolic pathways are active at different times of the 24 h day [8].*

Circadian rhythm and daily eating pattern regulate daily rhythm in metabolism. Cell autonomous circadian oscillators composed of transcriptional activators (CLOCK, BMAL1, NPAS2, ROR) and repressors (PER, CRY, REV-ERB) (henceforth called "clock components") are present in almost every organ [9]. The circadian oscillator in the hypothalamic suprachiasmatic nucleus (SCN) acts a master clock that generates daily rhythm in physiology and behavior such as activity and rest [10]. To adapt to this daily rhythm in activity-rest the circadian clock in different organs orchestrate daily rhythms in neuroendocrine factors that sense and signal energy states in different organs, rhythms in hunger and satiety hormones, and rhythms in function of hundreds of genes involved in catabolic and anabolic metabolism in organs such as liver, muscle, and adipose tissues [8, 11]. As a result, the circadian clock drives a daily cycle of feeding and fasting and orchestrates mechanisms to activate anabolic pathways to store nutrients during period of feeding and activate catabolic pathways to utilize the stored nutrients during period of fasting. Cellular metabolism produces reactive oxygen species (ROS) and molecular damage. Accordingly, circadian clocks also support a daily rhythm in cellular repair mechanisms including autophagy, mitochondrial fission-fusion cycle and ROS scavenging molecules [12-14]. Such rhythms in repair mechanisms sustain structural and functional integrity of organelles such as mitochondria [15].

To adapt to seasonal changes in day length, light entrains the SCN circadian clock to day:night cycle, which in turn adjusts the circadian clock in peripheral organs. However, the peripheral circadian clocks in metabolic organs including the liver responds to eating time, so that an abrupt change in eating time can reprogram the phase of the circadian clock and its output genes in the liver without affecting the SCN clock [16-18]. This "food-entrainment" of the peripheral clock may allow for rapidly adjusting the periods of anabolic and catabolic metabolism to the new timing of food availability, so that homeostasis is maintained.

Such acute effect of food on circadian rhythm forms the basis for understanding how daily cycles of feeding and fasting affect circadian gene expression and metabolic homeostasis. Systematic analyses of circadian gene expression in the mouse liver have revealed a dominant role of eating pattern on daily rhythms in gene expression. In the liver of fasted mice, only ~350 transcripts including the clock components and their direct output targets show daily oscillations. The normal pattern in mice when fed a normal chow *ad libitum*, is the consumption of the *majority* of calories at night when they are normally awake and their liver shows rhythmic expression of ~3,000 transcripts. Allowing mice to eat all of their food during their normal wakeful time only without altering caloric intake increases the number of rhythmic transcripts to ~5,000, which has a beneficial effect on metabolism. Conversely, (a) when mice eat randomly throughout the day and night, so that they consume more food when they are supposed to sleep, or (b) in mutant mice that lack clock components, daily rhythms in gene expression are dampened or abolished [8, 11]. These animals gradually succumb to metabolic diseases. In summary, these studies concluded that a daily rhythm in feeding and fasting supports a robust rhythm in numerous mRNAs and proteins, which regulate metabolic processes such as gluconeogenesis, glycolysis, protein synthesis, cholesterol synthesis, lipid synthesis or oxidation and mitochondria function.

Why are mRNA or protein rhythms beneficial? Majority of the genes that show daily rhythms in expression or activity play important roles in metabolism. Some of these key metabolic regulators include (cAMP-Response element-binding protein (CREB), activating transcription factor 6 (ATF6), mechanistic target of rapamycin (mTOR), forkhead box protein (FoxO), sirtuin proteins (SIRTs), sterol regulatory element-binding

protein (SREBP), and (peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) [19]. Furthermore, many of the clock components also directly regulate the expression of genes involved in nutrient metabolism [14]. *Daily rhythm in the function of these proteins with their peak activity timed to periods of fasting or feeding ensures the metabolic pathways they control are active only for a few hours every day when needed. For example, CREB mediated gluconeogenesis is active only for a few hours during fasting or mTOR pathways is active only during feeding. This leads to metabolic efficiency and ensures optimal function that supports normal health. Constitutive (constant) activation or constant suppression of these metabolic regulators is often found in metabolic diseases including diabetes, obesity, and fatty liver disease. Circadian clock mutant mice and animal models of chronic shiftwork show dampened gene expression rhythms and these animals are predisposed to metabolic diseases. Therefore, lifestyle or pharmacological interventions that improve daily rhythms in gene expression and function are expected to improve metabolic health.*

Circadian disruption increases risks for metabolic syndrome. Circadian rhythm disruption can be due to genetics (rare among humans) or lifestyle. Lifestyle induced circadian disruption is mostly due to (a) shiftwork - working (and staying awake) during habitual sleep time and trying to sleep during habitual wakeful hours, and (b) sleep deprivation – increase in the length of activity period. Shift workers, who account for >20% of the workforce, typically return to their daytime social life during off-days, and experience the internal drive for sleep and wakefulness. As the phase of circadian oscillator does not abruptly change in response to abrupt change in light:dark cycle as occurs in shiftwork, repeated resetting cues disrupt the normal circadian rhythm. A large body of epidemiologic and clinical evidence has indicated that shift work or sleep disturbances is associated with an increased risk of T2DM [8, 20-27]. Shift workers also experience erratic eating patterns. Erratic eating patterns, such as late night caloric intake, increase the risk of developing coronary heart disease by as much as 55%, even after controlling for diet and lifestyle [28]. Modeling shiftwork in animals disrupts the normal molecular circadian clock and predisposes them to metabolic diseases [29]. Even in non-shiftwork animal models, an erratic eating pattern causes circadian rhythm disruption and metabolic diseases [30, 31].

Aging animals and humans also show dampening of daily rhythms at neuroendocrine and physiological parameters [32] and the risk for metabolic diseases increases with age. Finally, in model organisms, genetic perturbation of the circadian clock abolishes their daily rhythm in activity and feeding and increases the incidence and severity of metabolic diseases [33-36].

In summary, these powerful observations in both animal models and humans support the premise that chronic circadian rhythm disruption can increase the risk for metabolic diseases. However, the underlying mechanism in most studies is confounded by the fact that chronic circadian disruption often accompanies sleep restriction, consumption of energy dense diets and/or erratic eating pattern. The proposed work will make key contribution to this knowledge gap by addressing the effect of altering duration of eating pattern on circadian regulation of metabolism.

Time-restricted eating: Time restricted eating (TRE) for 8-12 h - without reducing caloric intake relative to ad lib fed (ALF) controls – is an experimental feeding regimen that is effective in testing the role of daily feeding-fasting rhythm in circadian rhythms and in metabolic homeostasis. As mentioned earlier, ad libitum access to energy dense high fat diet dampens the molecular circadian oscillator [30], dampens the daily feeding rhythm [37], and is associated with obesity and metabolic diseases. Conversely, TRE of diet rich in carbohydrate, fat, fructose, or sucrose without reduction of caloric intake increases the robustness of circadian clock and can prevent or even reverse cardiometabolic diseases found in animals fed an identical diet ad libitum [38]. TRE exerts pleiotropic beneficial effects on multiple organ systems of mice (liver, muscle, white adipose tissue, brown adipose tissue, gut, and brain) [37-41] and insects (muscle, heart, brain) [42]. Mutant animals lacking circadian clock components do not exhibit the benefits of TRE, thus suggesting TRE acts through the circadian clock [42].

The most profound and well described effect of circadian rhythm disruption on metabolism in both rodents and humans is in glucose homeostasis. In healthy adults, glucose tolerance and insulin sensitivity exhibit diurnal rhythms [43-45]. **Circadian disruption, even for a few days, can cause insulin resistance or glucose intolerance [26, 27]**. In animals fed an obesogenic or high glycemic diet, TRE can restore normal glucose regulation [38]. The underlying molecular mechanisms of TRE restoring glucose homeostasis described in

rodents relates to restoring daily rhythms in mTOR and AMPK function. The anabolic mTOR pathway downstream of insulin is mostly active during the fed state, while the glucagon and AMPK-driven pathways leading to gluconeogenesis and fatty acid oxidation pathways are only active for a few hours during the habitual fasted state. Diet induced obesity or frequent eating causes both these pathways to be continuously active throughout the 24 h, which abolishes the circadian rhythm [37]. TRE restores normal circadian clock function and the daily rhythms in mTOR and AMPK function. In concert, TRE also restores normal levels and/or normal daily rhythms in several mRNAs, proteins, and metabolites that are implicated in metabolic homeostasis of lipids, cholesterol, redox and mitochondria function [37].

In summary, these observations suggest that erratic eating pattern or extended period of eating disrupts circadian rhythms and predisposes to metabolic diseases and TRE in animals restores these rhythms and prevents or reverses the diseases. This hypothesis will be tested in the proposed study by subjecting adults with metabolic syndrome who habitually eat over a >14 h duration to a 10 h eating window (with a 14 hour fasting period) and examining whether TRE improves glucose homeostasis.

TRE and mitochondria function. Mitochondria play a central role in nutrient homeostasis, and impaired mitochondria function and structure are common hallmarks of metabolic diseases in both humans and animals [46]. Conversely, many pharmacological and lifestyle interventions that improve mitochondria functions and structural integrity also alleviate metabolic diseases [47]. The circadian clock regulates several functions of mitochondria, and maintains its structural integrity [48]. Genetic or high fat diet induced circadian rhythm disruption compromises mitochondria function as well as structure [36, 48, 49] , which parallels development of metabolic diseases. The beneficial impact of TRE on mitochondria function in animals has been observed from molecular signatures to whole organism's fitness: gene expression, metabolites, mitochondria structure, mitochondria function, and physical activity. TRE selectively increases expression of PGC1α by 280%, a 2.8 fold increase [37, 38]; PGC1α is the master regulator of mitochondria biogenesis and of several mitochondrial proteins that function in the central metabolic pathways of mitochondria-such changes are observed at both the RNA and protein levels. Metabolomics analyses have revealed TRE increases the net metabolic flux through the mitochondrial Tricarboxylic Acid Cycle (TCA) and fatty acid oxidation [37, 38], which contributes to improved glucose homeostasis in these animals. TRE effects are conserved across species and are observed in *Drosophila*, where TRE led to reduction in expression of mitochondrial electron transport chain (ETC) components [42]. Reduction in ETC gene expression likely reduces the production of reactive oxygen species (ROS) and preserves mitochondrial structural integrity. Quantitative microscopy techniques have revealed mitochondrial volume and overall structure improves with TRE in rodents [37]. The improvement in mitochondria function is pervasive as it is observed in multiple organs in mice and Drosophila. These biochemical and ultrastructural improvement in mitochondrial function translates to improved physiology; TRE improves muscle function as measured by endurance in mice, flight ability and cardiac contractility in Drosophila [37, 38, 42].

While these animal studies clearly indicate TRE improves mitochondria function and structure in both liver and muscle tissues, whether circadian rhythm restoration through TRE can restore mitochondria function and structure in humans has never been explored. This proposal will directly test this hypothesis by examining gene expression, protein levels and structure of muscle mitochondria from subjects undergoing TRE.

Non-alcoholic fatty liver disease (NAFLD). Non-alcoholic fatty liver, which is defined as evidence of steatosis on imaging or histology, is highly prevalent, especially in developed countries, and is closely associated with obesity, insulin resistance and metabolic syndrome [65]. Metabolic syndrome and non-alcoholic fatty liver disease (NAFLD) both impact ~30% of the U.S. population, have related pathophysiology, and confer an increased risk of cardiovascular disease (CVD) [66-68]. Studies controlling for other CVD risk factors suggest a potential direct link between NAFLD and CVD risk, and CVD is the most common cause of death among patients with NAFLD. The putative mechanism linking NAFLD to CVD involves inflammatory and prothrombotic pathways, and derangements in lipid and glucose metabolism. Therefore, therapies designed to reverse NAFLD and metabolic syndrome may potentially contribute to prevention of cardiovascular, metabolic, and hepatic diseases [67]. First-line therapies for metabolic syndrome and NAFLD are diet and lifestyle interventions [67, 68]. Weight loss is a mainstay of therapy for NAFLD, and greater degrees of weight

loss have been associated with greater likelihood of NAFLD resolution. A randomized control trial of patients undergoing a comprehensive lifestyle intervention over 12 months showed that 97% of patients with ≥ 10% weight loss had NAFLD remission. The group undergoing intensive lifestyle intervention also had greater mean reductions in intrahepatic triglyceride content compared with control [69]. However, this lifestyle intervention was very aggressive, involving multiple sessions with dieticians with customized diet plans, exercise directed by a trainer, and close follow-up, making such an intervention potentially difficult to replicate in routine clinical practice with low long-term adherence. In mice fed a high fat diet, TRE results in reduced fatty acid synthesis as well as enhanced lipolysis. There is also significantly less hepatic steatosis in the livers of mice on TRE and a high fat diet, compared to those eating a high fat diet ad lib without TRE [70]. In mice fed a high fat diet, hepatic triglyceride content is significantly lower with 9 hours of time-restricted eating compared to ad lib eating ($p < 0.001$). There appears to be a dose-effect of TRE on serum triglyceride (TG) levels in mice fed a high fat diet: with ad lib eating mean TG = 179 mg/dL, but with 12-hours of TRE mean TG = 121 mg/dL, and with 9-hours of TRE mean TG = 73 mg/dL (ad lib eating vs 12- and 9-hours of TRE, $p < 0.05$ and $p < 0.01$, respectively). Mice fed a high fat diet ad lib also had greater relative abundance of serum cholesterol compared to mice on TRE: with ad lib eating mean cholesterol = 174 mg/dL and with TRE mean cholesterol = 94 mg/dL, $p < 0.001$ [71]. The effect of TRE on liver fat in patients with NAFLD has never been studied. Thus, additional studies examining the effect of TRE on hepatic fat and lipid metabolism in humans are needed. *There is an unmet need in NAFLD for lifestyle interventions such as TRE, which are simple, inexpensive and may have improved long-term adherence.*

8. PROGRESS REPORT

None.

9. RESEARCH DESIGN AND METHODS

Overall Study Design. Participants will be recruited from the cardiovascular and internal medicine clinics at UCSD and through a database search with EPIC, and we will register with www.clinicaltrials.gov. We plan to screen 5000 participants and enroll up to 400 to reach our randomization goal of 144 participants, age 18-75 years who meet criteria (refer to Section 10). Participants must have a self-reported dietary intake of ≥12 hour window, regular daytime schedule of activity (no shift workers) prior to study enrollment. The study will consist of a 2-week baseline screening period and then they will be randomized into one of two groups (standard of care or standard of care with TRE) for 12 weeks (14 weeks total). They will utilize the mCC app for the entire 14 weeks. Testing will include blood draw, anthropometric data, vitals, wrist-worn actigraphy device, muscle biopsy, and DXA scan before and after the intervention period. A subset of participants with NAFLD will have their intra-hepatic fat measured by MRI-PDFF before and after intervention.

Screening. Participants meeting eligibility criteria will be identified based on a database search through the UCSD patient medical record (EPIC), ResearchMatch, physician referrals, and flyers. A detailed phone interview will be conducted to further determine if the subject meets the inclusion and exclusion criteria. If participants pass this phase of screening, they then will be asked to come to the ACTRI to be enrolled and consented into the study at Visit 1. A "Visit 0" will be conducted if the subject does not have recent enough labs to determine their eligibility for the study. At this visit, all of the same procedures of Visit 1 will be performed, excluding the application of the actigraphy watch and continuous glucose monitor in order to ensure that a subject meets the inclusion criteria before these resources are used. If the results of the fasting blood work from the Visit 0 do qualify a subject, then they will be asked to come to the ACTRI for Visit 1 in which the actigraphy watch and continuous glucose monitor will be applied. They will not have to repeat the blood tests at this Visit 1. If the lab results disqualify a subject, they will be excluded from further study. Visit 1 will entail fasting blood work, general health questionnaires, and introduction of the mCCapp. The Beck Depression Inventory (BDI) will be used to screen for depression and the Epworth Sleepiness Scale (ESS) will be used to exclude for sleep apnea.

After reviewing the data from week 2, participants who record <2 meals/day for ≥3 days will be excluded from further study.

Randomization. The study statistician will generate the study randomization table prior to start of the study using SPSS program with block sizes of 4 and 6. He will be contacted by the study coordinator when a subject is ready to be randomized. 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. The statistician will be monitoring number of these groups and their randomizations to make sure there is an equal number in each study arm. To assess if this is a confounding factor, we will initially examine the treatment effect on these subjects separately and if our analyses indicate any significant additional source of variance (positive or negative), we will add household Status as a covariate to our analytical models. Their data will be combined with other subjects if there is no significant effect of their household randomization status.

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.

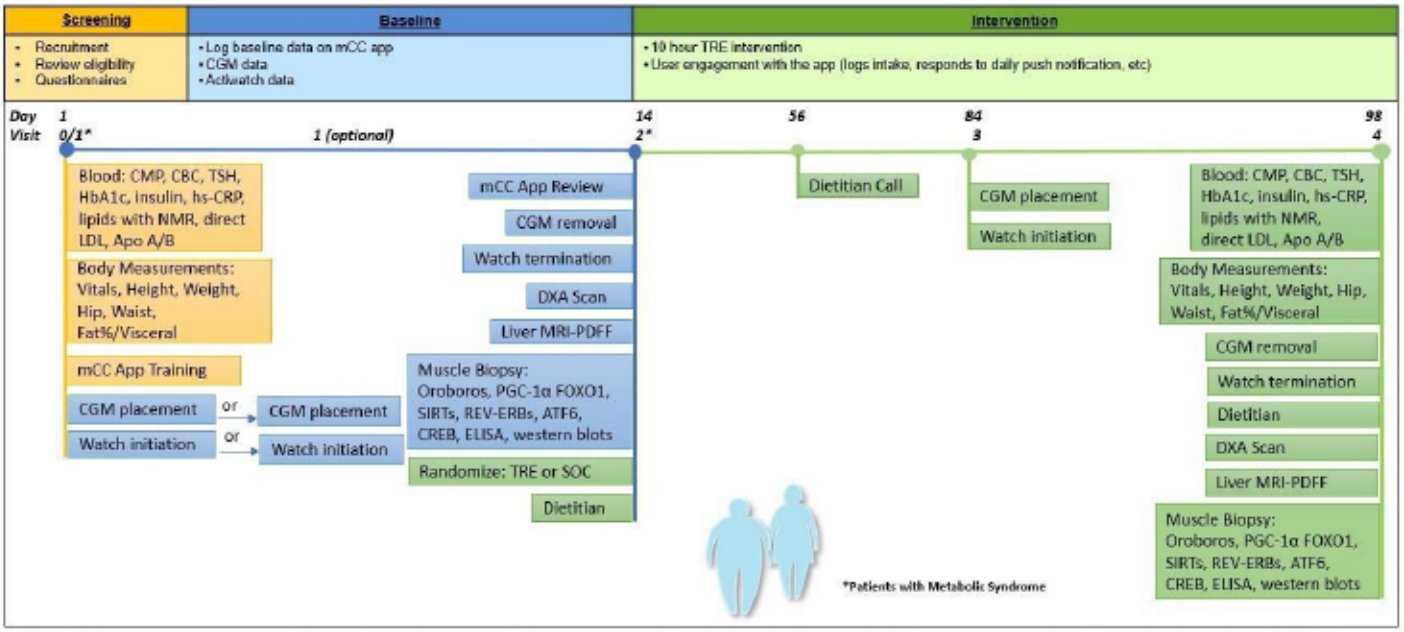

Figure 1. Timeline for participants. MCC app= myCircadianClock application. CGM= Continuous glucose monitor. DXA= Dual energy x-ray absorptiometry. TRE= Time-restricted eating. SOC= Standard of care. MRI-PDFF= magnetic resonance imaging derived proton density fat fraction and MRE. Visit 1, 2*= Participants will be disqualified if they do not meet inclusion criteria based on bloodwork, questionnaires, daily eating patterns, and adherence to the mCC app.

Visits (See Figure 1)

At visit 1 (start of 2-week baseline), participants will be consented, have a fasting blood draw and be asked to complete general health and sleep questionnaires. If the subject has NAFLD, they will be invited to undergo an optional liver MRI before and after intervention. When available, an existing study will be used as the baseline MRI-PDFF. The baseline laboratory tests for cardiometabolic function include the following: CMP = comprehensive metabolic panel, CBC = complete blood count, TSH = thyroid-stimulating hormone, HbAa1c= glycosylated hemoglobin, insulin, hs-CRP= high sensitivity C-Reactive Protein, Apo B/A =

apolipoprotein B/A ratio, TG = triglycerides, LDL, direct=Low density lipoprotein, HDL= high-density lipoprotein cholesterol, cholesterol assessed via NMR lipoprofile which will determine LDL Particle number. They will also be applied with the CGM and be given the actigraphy watch to wear for 2 weeks, unless their labs are not recent enough to determine their eligibility for the study, in which case the actigraphy watch and CGM will be not be applied until their lab results are reviewed and the subject does qualify for the study If a subject does qualify, these labs will be used as their baseline results. They will be introduced to the mCC app and instructed on its use. After visit 1, participants will enter a 2-week baseline period where they are instructed to use the mCC app to document all oral intake, exercise, and sleep quantity/quality.

At visit 2 (end of 2-week baseline), participants will return to the ACTRI to have their CGM and actigraphy devices removed. The mCC app will also be reviewed at this visit. Those who meet inclusion/exclusion criteria including logging criteria (recording >3 ingestion events of meals or beverages daily) and metabolic syndrome (based on blood work results, blood pressure, and waist circumference measurements) will be randomized into the standard of care group or TRE group. A muscle biopsy procedure and DXA scan will be performed at this visit as well for all participants regardless of group randomization. Each participant will meet with the dietitian to discuss his/her eating pattern for the subsequent 12 weeks. For the TRE intervention group, the coordinator and the participant will select an 8-10 h eating interval that best suits their lifestyle based on his/her baseline eating pattern. The length of this interval will be at least 4 hours shorter than their average eating window during their baseline, with a minimum eating window of 8 hours (from a 12-hour eating window baseline) and a maximum of 10 hours (from a baseline of 14-hours or greater). The interval will be entered in the app, so that they can visualize their daily eating pattern and consume all meals within this interval. Participants will also be able to modify the 8-10 h window based on their schedule (e.g. if they eat a late dinner out at 8 PM then they will be given instructions on how they can resume eating at 8 AM the following day). 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 >12h. Their mCC app will be programmed so that they can visualize their own caloric intake spread over the wakeful hours. Both groups will continue to use the mCC app to document all dietary intake, exercise, and sleep quantity/quality for at least 1 week every month.

Upon completion of visit 2, all participants will enter the 12-week intervention period. During this intervention period, they will either be engaged in the TRE group or in the standard of care group. During this time, participants of both groups will meet with the dietitian over the phone once during week 8 to reinforce good nutritional practices. In addition, participants will receive push notifications on their phones (once daily) which will ask them to indicate whether they have eaten anything in the past 15 minutes (from time of response to the push notification). The purpose of these push notifications is to assess the false negative rate (food eaten but not documented), which accounted for 10.34% in the pilot study involving use of this smartphone app [50]. At visit 3 (10 weeks into the 12-week intervention period), participants will return for the application of CGM and the actigraphy device for two weeks. At visit 4 (last week of intervention), participants will visit the ACTRI to have their CGM and actigraphy device removed. At this final visit, they will also have a fasting blood draw and be asked to complete the same general health and sleep questionnaires from visit 1. They will meet with the study dietitian for the final time. A muscle biopsy procedure and DXA scan will be performed at this visit. Additionally, if they are in the NAFLD sub-group, they will complete the liver MRI at this visit. This will conclude the 12-week TRE intervention for the 14-week study period (Fig 1).

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 8-10 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, muscle biopsy, and/or DXA scan at these optional follow-up visits.

Upon completing the study (or upon withdrawal) to avoid study bias, subjects will receive the results from the watch, CGM, DXA, and muscle biopsy data as an email attachment or printout. They will not be uploaded to the EMR. The lab results will be uploaded to the EMR as they are obtained from the lab. Subjects will already have access to the app data.

Procedures.

myCircadianClock application ("mCC app"). As it is increasingly becoming clear that the daily organization of activity, rest and eating pattern affect the circadian system and is also an output of circadian rhythm, longitudinal monitoring these parameters over days would produce important insight into circadian organization in humans. Co-I Panda has developed a smartphone app that combines both sensor-paired and self-reported logging of food, sleep and activity. The mCC app (see figure 2) 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 two different arms (TRE and Control). After randomization, the coordinator will assign a participant to one of two arms and generate a unique activation code. Subjects in the TRE arm can set their daily eating periods and receive alerts and reminders specific to TRE protocols. The TRE groups will receive an automated alert 15 or 30min prior to the end of the eating interval to finish their last meal of the day. In the control cohort, the daily eating period customization will be disabled and they will receive standard of care prompts. All groups 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 from each cohort. For food entries, the user can annotate the food picture with food name and any other descriptor (portion size, left over etc.). Each cohort will be asked to record all food intake for at least one week every month for the duration of the study (14 weeks). Unlike other food tracking app, where each food logging takes an average of 40 seconds (myFitnessPal or Noom), the mCC app takes less than 10 seconds for logging one meal by using the picture feature. The simplicity of logging food metadata has led to good adherence with the app. Due to the fact that the app is only available in English, subjects are required to be fluent in English to properly use and read the information in the app.

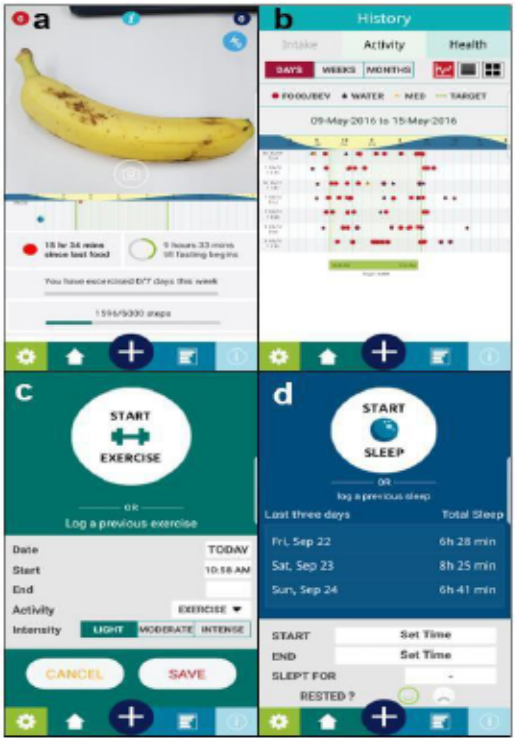

Figure 2. Overview of the mCC app

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).

Continuous glucose monitoring (CGM). All participants will be fitted with an Abbott Freestyle LibrePro® CGM, and be instructed on its use. CGM measures interstitial fluid glucose every 15 min for 14 full days, using subcutaneous sensor placed in the upper arm area. They will wear the CGM for two weeks prior to the start of the 12-week intervention period, and for two weeks prior to the end of the 12-week intervention. CGMs estimate

blood glucose levels with high accuracy that correlates with those obtained from either venous or capillary blood [51]. Changes in the post-prandial glucose response (PPGR), mean amplitude of glycaemic excursion (MAGE), continuous overall net glycaemic action (CONGA), the glycaemic variability index (GVI) and standard deviation (SD) will be calculated. Average daily glucose level will be the primary endpoint.

Wrist-worn actigraphy. To measure habitual physical activity and sleep, participants will wear a Phillips accelerometer on their non-dominant arm for two weeks during week 1 and week 12. Wrist accelerometers have acceptable correlation (r=0.90) between daily physical activity and activity counts reported by accelerometers [52] and have been used reliably in NHANES [53]. The actigraphy data will be used to measure sleep onset, sleep duration and associated parameters (sleep efficiency and sleep fragmentation). Participants will also complete a short sleep log every morning on the mCC app to record self-reported quality of sleep.

Cardiometabolic biomarkers. All participants will have their fasting blood drawn at visit 1 and visit 4 at the ACTRI by certified nurses. The baseline laboratory tests for metabolic function (conducted at visit 1) include the following: CMP = comprehensive metabolic panel, CBC = complete blood count, TSH = thyroid-stimulating hormone, HbAa1c= glycosylated hemoglobin, insulin, hs-CRP= high sensitivity C-Reactive Protein, TG = triglycerides, LDL=Low density lipoprotein, HDL= high-density lipoprotein cholesterol, direct LDL, Apo B/A = apolipoprotein B/A ratio, cholesterol assessed via NMR lipoprofile which will determine LDL Particle number. An aliquot will be stored in peptidase and phosphatase inhibitor coated tubes for subsequent analyses of peptide hormones by multiplexed ELISAs. A total of approximately 1 cup (200ml or 16 tablespoons) will be collected from the subject for the entire study. There is the possibility of collecting more if the blood tests do not result due to lab and/or processing errors.

A blood repository will be built. Blood samples (plasma, serum, whole blood) collected during the study will be stored and investigated at UCSD or the Salk Institute for the purposes of the study and also for possible future unforeseen biomarker testing such as neuroendocrine, inflammatory, and/or cardiometabolic testing. There is no intention to do genetic testing. Dr. Pam Taub will have solitary control of the stored specimens, which will be stored for an indefinite period of time. The samples will be labeled with a unique subject code as to de-identify and remove any PHI from the sample. The samples will be analyzed after subject completes study, and the analysis will be conducted in batches based on availability of resources. Samples will not be shared with researchers outside of those associated with this protocol.

Meals, activity and sleep. The smartphone application myCircadianClock (mCC app) will serve as an electronic food, activity, and sleep diary. On the server side, a sub-study dashboard will be created for this specific project. Clinical coordinators from Dr. Taub's lab and a research associate from Dr. Panda's lab will have password-protected access to the study data. In the study summary dashboard, the participant's study code and the date of activation of the app will be shown along with their daily log. If a participant fails to log any food data for more than 1 day, the dashboard flags the participant and sends an alert to the coordinator. The coordinators will login to the dashboard weekly to monitor food intake data, and follow up with flagged participants as necessary. The TRE participants will self-select a 10 h interval which they will consume all of their food and beverages. Their chosen eating interval is highlighted in the app so that they can easily track the progress of their daily eating within their set interval. For the standard of care groups, the participants' eating interval will be set to an eating pattern of >14 h. If any participant faces difficulty logging data, or has questions about any of the features of the app, they can contact the study coordinator through the feedback feature of the app. Their questions will be encrypted and delivered to a HIPAA compliant email server specifically set up for this study.

Non-invasive assessment of liver fat using magnetic resonance imaging proton density fat fraction (MRI-PDFF) and magnetic resonance elastography (MRE) While there are many non-invasive methods of

assessing liver fat, MRI-PDFF is considered the best non-invasive end-point for assessing changes in intra-hepatic triglycerides in clinical trials. This method provides a validated, accurate, and reproducible quantification of fat over the entire liver and is considered to be an appropriate surrogate for liver biopsy to assess response to treatment in clinical trials [72]. MRI-PDFF has the power to detect small, but meaningful changes in intra-hepatic lipid and performed better than biopsy in this respect in a trial examining the effects of colesevelam on hepatic fat [73]. In the NAFLD Research Center at UCSD, we have the necessary infrastructure and strong expertise to perform MRI-PDFF, as outlined in this proposal. Participants will be asked to change into a medical gown and lay still on a table for 60 minutes during the scan. Liver fat will be measured and changes in levels will be recorded. MR images for MR elastography will be obtained after placing a vibrating paddle over the abdomen during the liver MRI.

Dual Energy X-ray absorptiometry (DXA) scan. A total of two DXA scans will be performed at the ACTRI, once at the beginning of the study before the intervention period and once at the end of the study after the intervention period. DXA body composition will be measured with a Hologic Discovery W densitometer, quantifying fat mass of the total body, arms, legs and trunk, abdomen, and hip. Participants lie supine on a table for six minutes during the scan. Changes in body composition such as fat and muscle mass will be documented.

Muscle biopsy. Under local anesthesia, we will use a 5mm side-cutting needle for the percutaneous muscle biopsy. We will obtain 200-300mg of skeletal muscle from the lateral portion of the quadriceps femoris (vastus lateralis). A portion of the tissue will be immediately frozen in liquid nitrogen for subsequent RNA and protein extraction. A portion will be fixed in tissue fixative for microscopy analyses following a light microscopy technique optimized for muscle mitochondria [54] at the Salk Institute Waitt Biophotonics center using a ZEISS LSM 800 with Airyscan. Four different thin muscle sections from each sample will be imaged and analyzed. A stereological analysis to ascertain the ratio of mitochondrial volume to cytoplasmic volume will be performed. Point counting will be used to determine the mitochondrial volume densities by overlaying a grid on each digitized image. Mitochondria and cytoplasm lying under intercepts will be counted. The relative volume of mitochondria will be expressed as the ratio of intercepts coinciding with this organelle to the intercepts coinciding with cytoplasm. Mitochondrial membrane surface areas are to be measured using Image J software. Sample preparation, imaging and data processing will be performed by trained microscopy technicians at the Waitt Biophotonics Center.

Muscle samples collected during the study will be stored and investigated at UCSD or the Salk Institute for the purposes of the study and also for possible future unforeseen mitochondrial testing. There is no intention to do genetic testing. Dr. Pam Taub will have solitary control of the stored specimens, which will be stored for an indefinite period of time. The samples will be labeled with a unique subject code as to de-identify and remove any PHI from the sample. The samples will be analyzed after subject completes study, and the analysis will be conducted in batches based on availability of resources. Samples will not be shared with researchers outside of those associated with this protocol.

RNA analyses. The muscle will also be analyzed for changes in gene expression levels in Dr. Panda's lab at Salk. As observed in animals, TRE is hypothesized to change the expression or function of key transcriptional regulators of mitochondria biogenesis and function (PGC-1α FOXO1, SIRTs), lipid metabolism (REV-ERBs), protein folding (ATF6), and glucose metabolism (CREB). Furthermore, hypothesized changes in mitochondria number and function would reflect in changes in expression of key nuclear encoded mitochondria genes. Therefore, qPCR offers an inexpensive method to simultaneously quantify muscle gene expression. Additionally, some of the mitochondrial functional analysis will be done in the lab of Dr. Hemal Patel at UCSD.

Results will be validated by western blot analyses of protein levels. Total RNA will be extracted using RNeasy columns (Qiagen), following the manufacturer's instructions. Total RNAs from all samples will be processed in a single batch to reduce experimental noise. Reverse transcription followed by quantitative PCR

(RT-qPCR) protocols using gene specific validated primer pairs will be used in triplicate reactions. Data normalization and quantification will be done using procedure followed in the Panda lab [55].

Protein analysis. Muscle protein will be extracted following standard protocol and protein levels will be quantified by multiplexed ELISA, or western blot using antibodies targeting specific markers of muscle or mitochondria function. Mitochondria function will be analyzed by citrate synthase activity using the Srere method [56].

Tissue Sample Analysis.

Tissue fixation for transmission electron microscopy: 25mg of muscle tissue will be places in 4% paraformaldehyde and 1.5% glutaraldehyde in 0.1 M cacodylate buffer and then will be postfixed in 1% OsO4 in 0.1 M cacodylate buffer, en bloc stained with uranyl acetate, dehydrated through standard ethanol series, and then embedded in longitudinal orientation embedded in LX-112 (Ladd Research, Williston, VT, USA). Blocks will be polymerized 48 hours at 60°C. 70nm sections will be cut and mounted on Cu grids, stained in uranyl acetate and lead citrate prior to TEM analysis (JEOL JEM-1400Plus transmission electron microscope equipped with a 4K Gatan OneView digital camera, and the observes blinded to treatment regimen.

Mitochondrial Respiration on Oroboros Direct from Tissue: 1-3mg of fresh tissue will be utilized for direct assessment of mitochondrial respiration using standard protocols for fatty acid utilization and mitochondrial complex function using our OROBOROS system. The assays probe electron transport chain function and capacity using three primary parameters: fatty acid metabolism and specific assessment of complex I and II function, maximum oxidative capacity, and maximum uncoupled capacity. The laboratory currently has 7 O2K systems each with 2 ports for analysis giving 14 effective chambers. Five of these systems are also equipped with LED fluorescent modules that allow simultaneous measurement of ATP, mitochondrial membrane potential, and ROS generation coupled with mitochondrial respiration. The system is primarily used for tissue giving high relevance to recording from our human samples, but can also reliable measure function in isolated cells and mitochondria.

Blood/Plasma Sample Analysis.

Electron Paramagnetic Resonance (EPR)-Ascorbyl Radical

50uL of plasma will be loaded into a capillary tube and subjected to a spectral scan using the EPR system. The scan results in a spectral signature that can be quantified for ascorbyl radical as a global measure of oxidative stress. This can be monitored in serial blood collections.

Seahorse Mitochondrial Respiration Assay Using Plasma

Oxygen consumption rates (OCR) from a monolayer of adherent L6 muscle cells will be measured using the Seahorse XF96 analyzer. The L6 cells will be seeded at 20,000 cells/well (passage 3-4) on a 96 well tissue culture plate (Seahorse Biosciences, North Billerica, MA, USA) 24h before the OCR measurements. The intact cells will then be incubated at 37oC for 1h with XF media supplemented with 10mM glucose, 1mM sodium pyruvate, 2mM L-glutamine, pH 7.4. The bioenergetic profile will be derived by acutely injecting control or patient plasma (5%) through the port A followed by the modulators of the electron transport chain in form of drugs such as Oligomycin (1uM) to inhibit ATP synthase, carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP) (2uM) for maximal uncoupling and Rotenone/Antimycin A (0.5uM), a complex I inhibitor. The OCR will be calculated by plotting the O2 tension of the media as a function of time (pmol/min). The assay can be expanded to assess plasma effects on cardiac, neuronal, immune, kidney, and liver cell.

Isolation of Peripheral Blood Mononuclear Cells

20-30mL of whole blood is collected in BD Heparin Tubes (BD 366480). The blood is subjected to a Ficoll gradient for separation of PBMCs. The isolated PBMC fraction is collected and plated into Seahorse plates and subjected to the assays described above for assessment of mitochondrial respiration. PBMCs mitochondrial function is a surrogate and peripheral marker indicative of tissue level metabolism. [57][58]

Statistical Analysis.

Specific Aim 1 – Evaluate the impact of TRE on glucose homeostasis.

Hypothesis 1: The TRE group will significantly decrease HbA1c compared to the control group at the end of the study.

Independent Variables: Group (TRE, Control Group), Week (with two levels of Baseline Week and Intervention Week), Day (with 7 levels of days 1-15 for Baseline Week and days 84-98 for Intervention Week).
Dependent Variables: HbA1c.
Statistical Analysis: HbA1c data will be analyzed to determine changes in glucose response within individuals. Initially preliminary analyses will be performed for all hypotheses by examination of the distribution of variables to assess their characteristics (means, standard deviations, and skewness). Continuous measures will be tested for normality and homogeneity of variance. Non-normally distributed variables will be transformed to meet the normal distribution assumption for linear models. Randomization will be assessed by performing a series of Wilcoxon-rank sum tests, Chi-square or Fisher's exact tests to compare the groups on demographic and initial baseline clinical variables. Any variables on which the groups differ initially will be explored as covariates in subsequent analyses. A daily average for glucose value will be computed for each week of study (total of 14 daily measurements). This hypothesis will be tested by using mixed effect model as described by Gibbons et al (1988) [59], Hedeker et al (1991) [60] and Laird et al (1982) [61]. The model will include a random intercept and random effect for Week and Day factors. A fully saturated treatment by Week by Day model will be utilized for inference. Co-variance structure will be chosen based on Akaikes Information Criterion (AIC). Denominator degree of freedom will be calculated using the Kenward-Roger small sample correction. The primary outcomes will be tested using an intent-to-treat framework and no subjects will be excluded from the analyses. Data will be analyzed using SPSS version 24 (or current version). All analyses will be two-tailed, where applicable, with $\alpha = 0.05$.

Sub-aim 1A: Assess changes in other glycemic measures including fasted blood glucose, insulin, HOMA-IR and continuous glucose monitors (CGM).
Hypothesis: The TRE group will have a significant improvement in glycemic parameters (assessed via fasting glucose, HOMA-IR, Insulin, and CGMs) compared to the control group and compared to baseline.

Independent Variables: Group (TRE, Control Group), Week (with two levels of Baseline Week and Intervention Week), Day (with 7 levels of days 1-15 for Baseline Week and days 84-98 for Intervention Week).
Dependent Variables: Fasting glucose, Fasting Insulin, HOMA-IR, and glycemic control assessed via CGM (including mean glucose, time-in rage, mean glucose while fasting or fasting, and mean glucose night or day)
Statistical Analysis: Data will be examined initially similar to hypothesis 1. This hypothesis will be tested using Repeated-Measured Analysis of Variance.

Specific Aim 2 – Assess changes in cardiometabolic biomarkers in response to TRE.
Hypothesis 2: TRE induced decreases in blood glucose levels will be accompanied by improvements in cardiometabolic biomarkers at the end of the study.
Independent Variables: Group (TRE, Control Group), Day (Baseline: day 1 and Intervention: day 98).
Dependent Variables: Cardiometabolic Biomarkers
Statistical Analysis: Data will be examined initially similar to hypothesis 1. This hypothesis will be tested using Repeated-Measured Analysis of Variance. All analyses will be two-tailed using Bonferroni correction method for multiple testing.

Specific Aim 3- Assess the impact of TRE on body composition

Hypothesis 3: The TRE group will have significantly lower abdominal fat mass compared to the control group at the end of the study.
Independent Variables: Group (TRE, Control Group), Day (Baseline: day 15 and Intervention: day 98). Dependent Variables: *DXA*.
Statistical Analysis: Data will be examined initially similar to hypothesis 1. This hypothesis will be tested using Repeated-Measured Analysis of Variance.

Exploratory Aim 1 – Elucidate the effects of TRE on changes in mitochondrial structure and gene expression in skeletal muscle.

Impaired glucose homeostasis can result from insulin resistance, compromised mitochondrial function, adiposity, dyslipidemia, and inflammation. To elucidate the underlying mechanisms, skeletal muscle biopsies will be utilized to analyze 1) alterations in mitochondrial structure/function and 2) changes in gene expression of candidate target genes triggered by TRE. These exploratory analyses will be performed using Mixed effect model or RM-ANOVA.

Exploratory Aim 2 – Evaluate for change in hepatic steatosis (liver fat), quantified using magnetic resonance imaging derived proton density fat fraction (MRI-PDFF), after 12-weeks of TRE.

High levels of hepatic fat are closely associated with cardiovascular disease risk markers such as obesity, insulin resistance and metabolic syndrome. Reduced liver fat could serve as an imaging marker that heralds reduction in cardiovascular and liver disease risk with TRE in this population. To assess whether there is a reduction from baseline intra-hepatic lipid content with TRE, MRI-PDFF scans will be utilized. This will be analyzed using paired samples t-test (two-tailed, $p < 0.05$ considered significant) comparing pre- vs post-TRE % liver fat by MRI-PDFF.

Missing Data: Missing data values will be minimized by intensive training of the interviewers in techniques of clarifying answers and checking questionnaires while participants are on-site. When missing values are identified, we will employ several approaches. If at all possible, participants will be rescheduled or a tester sent to the individual's place of residence within 24 hours of completion of tests or interviews. Missing data will be examined to assess randomness. The pattern of missing data will be examined according to the procedure recommended by Little and Rubin [62] which includes comparing group differences in the primary outcomes of subjects with versus without missing data. The selected method of data analysis allows inclusion of subjects with missing data or those who terminated the study early, without relying on data imputation procedures. All laboratory-based measures will be reviewed for completeness immediately following collection and prior to the participant's dismissal. Despite our intensive protocol to limit participant attrition, we do expect missing data over the course of this study and have adjusted our recruiting sample accordingly. The primary outcomes will be tested using an intent-to-treat framework. If the extent of missing data is small and the data appear to be consistent with a missing-at-random model (MAR), then the maximum-likelihood analysis using all randomized cases and the observed data is an appropriate method for handling the missing data. In the MAR model the missingness can be a function of the observed covariates and observed outcomes. The MAR assumption is effective in treatment situations. The critical element when conducting MAR-based analyses is to include variables related to the missingness in the statistical model. The distinction between ignorable (MAR) and non-ignorable missingness (missing not at random; MNAR) is generally not empirically testable and we acknowledge the possibility that data may be missing not at random. Therefore, we propose to perform MNAR sensitivity analyses using pattern mixture models [63]. Some patterns will likely have small numbers of cases precluding parameter estimation for these patterns separately. In this case we will apply the Hedeker and Gibbons approach [64] that combines missing data patterns to estimates model parameters that are conditional on the missingness using a binary variable in the model to denote missing data at one or more time points.

We will also test whether the drop-outs are random or systematic by comparing the drop-outs with the study completers on the baseline data. An absence of significant differences would support the random nature of drop-

outs. Effect of drop-outs will be minimized by the following steps: First, we had selected data analysis method which allows the inclusion of subjects who drop-out or were terminated early in the study, without relying on data imputation procedures. Therefore, no subjects will be excluded from the analyses due to their study status. Second, effect of any systematic differences between the drop-outs and completers will be explored by including the variable of concern to the model as a covariate.

Power Analysis: The required sample size was calculated using G*Power software and for the mixed model approach using the RMASS program provided by Hedeker (http://tigger.uic.edu/~hedeker/ml.html). We are confident that the proposed sample size of 118 provides us with a minimum power of 80% to detect a medium effect size of 0.55 for all hypotheses. Medium effect sizes were selected based on previous studies that are reviewed above (see pages 4-5) and fasting glucose data from 12 participants who had elevated glucose from our pilot study. For the mixed model, the medium effect size is defined as a between-group difference increasing linearly from 0 at baseline to .5 SD units at the last time point. The minimum power estimation is based on sample size calculation for 10% and 20% attrition, correlations of 0.2, 0.5, and 0.8 between the repeated measures, and for medium and large effect sizes.

Data Management: Data management and statistical analysis will be performed by the study statistician, Dr. Shahrokh Golshan. A comprehensive menu driven data management system will be developed for this project using Microsoft Access software. This system will provide a stable and secure platform for: a) study data input, validation, edit and query; b) subject tracking system for all subjects' study activities (screening and study visits); c) data storage, compilation, selection, and summary transfer to various statistical/graphical software and for institutional reports (IRB, DSMB); and d) data backup and archival control. All subjects are assigned a unique ID number. The patient names are never used in conjunction with the unique clinic ID number. The single name-to-ID relational file is kept in an encrypted form electronically and in a locked filing cabinet physically. Statistical analysis will be conducted using SPSS software package version 24. All statistical transfer routines are inherently secure via their operating platform and they contain no patient names or personal data.

10. HUMAN SUBJECTS

In order to randomize 118 adult volunteers (59 in the TRE arm and 59 in the control arm), we expect to screen 5000 participants. . To define the terms enroll and screen, we will screen 5000 participants via EPIC chart screening and enroll (consent) up to 400 participants. Our goal is to randomize 144 participants, and to do that, we will enroll up to 400 subjects until this goal is reached. We anticipate a 20% drop-out rate with 94 participants completing the trial. We may randomize up to 144 participants (72 in the TRE arm and 72 in the control) to compensate for dropouts if needed. We will recruit patients from ResearchMatch and the cardiology and internal medicine clinics at UCSD Medical Center; in addition, we will utilize the bioinformatics report generated from EPIC to assist in recruitment. Participants will be screened and must meet inclusion and exclusion criteria before they are enrolled in the study. The inclusion and exclusion criteria are below.

Inclusion criteria:

1. Age: 18-75 years
2. $41 \geq BMI \geq 25$ AND
3. metabolic syndrome, as defined as presence of 3 or more of the following criteria:
    - Elevated fasting plasma glucose $\geq$ 100 mg/dL and/or $HbA_{1c} \geq 5.7\% < 7.1\%$
    - Elevated waist circumference:
        - In Asians: $\geq$ 90 cm in men, $\geq$ 80 cm in women
        - In all other races: $\geq$ 102 cm in men, $\geq$ 88 cm in women
    - Fasting plasma triglycerides $\geq$ 150 mg/dL, or on drug treatment for elevated triglycerides

- Reduced High-density lipoprotein (HDL)-cholesterol <40 mg/dL in males or <50 mg/dL in females, or drug treatment for reduced HDL-cholesterol
- Elevated blood pressure, Systolic blood pressure ≥ 135 mm Hg and/or diastolic blood pressure ≥ 85 mm Hg or drug treatment for hypertension

4. Own a smartphone (Apple iOS or Android OS)
5. Baseline eating period ≥ 12 hour window
6. 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-hypertensive, anti-diabetes drugs), no dose adjustments will be allowed during the study period.
7. For participants with NAFLD who agree to participate in the optional liver MRI: baseline percent intra-hepatic fat ≥ 5% by magnetic resonance imaging derived proton density fat fraction.

Exclusion criteria:

1. Taking insulin within the last 6 months.
2. Manifest diabetes, defined as $HbA_{1c} > 7.0\%$ given a 0.3% margin of error in lab readings, or diagnosis of diabetes.
3. Known inflammatory and/or rheumatologic disease.
4. Active tobacco abuse or illicit drug use or history of treatment for alcohol abuse.
5. Pregnant or breast-feeding women.
6. Shift workers with variable (e.g. nocturnal) hours.
7. Caregivers for dependents requiring frequent nocturnal care/sleep interruptions.
8. Planned travel to a time zone with greater than a 3-hour difference during study period.
9. History of major adverse cardiovascular event within the past 1 year (acute coronary syndrome (ACS), percutaneous coronary intervention, coronary artery bypass graft surgery, hospitalization for congestive heart failure, stroke/transient ischemic attack (TIA)).
10. Uncontrolled arrhythmia (i.e. rate-controlled atrial fibrillation/atrial flutter are not exclusion criteria).
11. History of thyroid disease 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).
12. History of adrenal disease.
13. History of malignancy undergoing active treatment, except non-melanoma skin cancer.
14. Known history of type I diabetes.
15. History of eating disorder(s).
16. History of cirrhosis.
17. History of stage 4 or 5 chronic kidney disease or requiring dialysis.
18. History of HIV/AIDS.
19. Currently enrolled in a weight-loss or weight-management program.
20. On a special or prescribed diet for other reasons (e.g. Celiac disease).
21. Currently taking any medication that is meant for, or has known effect on, appetite.
22. Any history of surgical intervention for weight management.
23. Uncontrolled psychiatric disorder (including history of hospitalization for psychiatric illness).
24. A score of >16 on the Epworth Sleepiness Scale (ESS).
25. Depression determined by the Beck Depression Inventory (BDI) (unless previously diagnosed and well-controlled)
26. Failure to use the smartphone app for documentation (defined as <2 meals/day for ≥3 days during baseline).

The main site of the interviews and clinical testing (vitals, blood draw, muscle biopsy, DXA scan) will be at the UCSD Altman Clinical and Translational Research Institute (ACTRI). Blood will be processed at the UCSD Clinical Laboratory before being transferred to Salk institute for storage and analysis.

11. RECRUITMENT AND PROCEDURES PREPARATORY TO RESEARCH

We will screen and recruit normal subjects and patients from UCSD. We will also utilize ResearchMatch. We will also utilize social media to advertise for the study (for example, the generation of a Facebook ad). We will also recruit participants from UCSD primary care clinics using methods identical to those being implemented under HRPP protocol #130554. Specifically, primary care patients that are shown to meet our eligibility criteria as part of their routine clinic visit will be sent an HRPP-approved recruitment letter electronically for UCSD patients registered for 'MyUCSDChart' which provides private and secure online access to health information. This letter will be sent automatically as a MyChart message, which will be from Dr. Bill Sieber. If a participant contacts us and expresses interest in the study, we will then contact their primary care physician to ensure their approval of their patient's participation in the study.

We will also record a video of participants (with their consent) discussing their experience in the study, which will be posted on our research website and may potentially be emailed to potential participants as well. We will also be screening patients through EPIC, with the help of their bioinformatics department, for research subjects who might qualify for our criteria. EPIC Bioinformatics will help search for the following to generate a list of potential research subjects:

1. Patient's name, date of birth (DOB), and Medical Record Number (MRN)
2. Patient's primary care provider (PCP) name and contact information
3. Ages 18-75 for inclusion.
4. $41 \geq BMI \geq 25$ for inclusion.
5. Metabolic Syndrome:
    a. Waist circumference:
        i. In Asians: ≥ 90 cm (Men) or ≥ 80 cm (Women) for inclusion.
        ii. In all other races: ≥ 102 cm (Men) or ≥ 88 cm (Women) for inclusion.
    b. Triglycerides ≥ 150 mg/dL (or on drug treatment for elevated triglycerides) for inclusion.
    c. Reduced HDL-C < 40 mg/dL (Men), < 50 mg/dL (Women) (or on drug treatment for reduced HDL-C) for inclusion.
    d. Elevated blood pressure, systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg (or treatment with antihypertensive drug with history of hypertension) for inclusion.
    e. Elevated fasting glucose ≥ 100 mg/dL and/or $HbA_{1c} \geq 5.7\% < 7.1\%$ (or drug treatment of elevated blood glucose) for inclusion.
6. No prior adverse cardiovascular events (ex: heart attack) for inclusion.
7. No prior cerebrovascular events (ex: stroke) for inclusion.
8. History of liver or kidney disease for study exclusion.
9. History or chronic infectious disease; autoimmune/inflammatory disease, cancer, COPD, anemia, type I diabetes, familial hypercholesterolemia, history of weight loss surgery, history of venous thromboembolism, cancer, malignancy, or psychiatric illness for study exclusion.
10. Depression or sleep apnea for exclusion.
11. History of smoking and/or illicit drug use and/or treatment for alcohol abuse for study exclusion.
12. Currently pregnant, for exclusion.

After we identify patients of interest for the study, we will contact their primary care provider or cardiologist and provide them with a letter with the following information: brief study description, research team contact information, and request to approach/contact subject. We will also ask the clinicians to specifically ask the

interested patients if it is acceptable if one of our research team contacts them. If the patients are interested and give their consent, then our study coordinator will either contact them by email, phone, or clinic visit.

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 personal health information (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 research office at the Altman Clinical Translational Research Institute (ACTRI). 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 metabolic syndrome.

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 (see

section 21), 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 metabolic syndrome.

Pre-Screening/Recruitment

A waiver of documented consent for recruitment purposes (pre-screening only) is being requested. The pre-screening part of the research is minimal risk, and the waiver will not adversely affect the rights and welfare of the subjects. As this waiver is only being requested for pre-screening purposes, no study-related procedure or data will be collected prior to the actual consenting. In order to enroll the correct patient population, it is critical to review the medical records and ask questions regarding the inclusion/exclusion criteria of the potential subjects as much as possible in order to prevent enrollment of subjects who do not qualify into the trial. This will provide adequate time for the investigators to review all the relevant records prior to enrolling subjects. Upon reviewing records and eligibility criteria, all the potential subjects who might qualify for the trial and are still interested in the trial will be presented with the actual informed consent form to enroll.

Reconsenting

In the event that the consent is amended, subjects who are active and have not yet completed the study will be informed of these changes and be asked to sign the updated consent form as required by the IRB. The updated consent form will be provided to them at an upcoming visit, or through mail, email or fax. They will be given time to review these changes and provide their written consent. They may sign and return it to the research team at the upcoming visit, or through mail, email or fax.

Subjects who have completed the study and are no longer active in the study will be made aware of the changes through mail, email or fax. However, these subjects will not be asked to acknowledge or confirm the receipt of the updated consent or to sign and return it.

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. 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. Dual energy x-ray absorptiometry (DXA) 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 DXA 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 your 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.

8. Magnetic resonance imaging/ magnetic resonance elastography: Patients will be screened for and excluded if they have an implanted devices or metallic objects which increase the risks associated with MRI (MR Unsafe devices/objects). Such devices include certain intracranial aneurysm clips, cochlear implants, prosthetic devices and pacemakers. MR Safe devices will be allowed, and MR Conditional devices will be reviewed on a case-by-case basis by the Principal Investigator. Final decisions regarding whether a patient may undergo MRI will be made by the Principal Investigator, who will provide counseling to all patients prior to undergoing MRI. Other risks associated with MRI include anxiety, sensitivity to loud noise, and claustrophobia. We will not use any intravenous contrast agent for these MRI studies. MRE is a type of MR imaging in which the images indicate tissue stiffness. MRE imaging involves placing a vibrating paddle over the abdomen while images are being obtained. This is an FDA-approved procedure when used clinically, but is considered to be investigational in this study. These vibrations have been reported to be well tolerated by patients. However, the vibrating paddle could be uncomfortable to some subjects, and so subjects will be instructed to tell the MR technologist if the vibrations become uncomfortable, and the MRE part of the examination will be discontinued.

9. Muscle Biopsy: The risks of the muscle biopsy include vasovagal events, pain, bleeding, bruising and infection at the biopsy site. These risks will be minimized with pressure and bed rest after the procedure. The risks of using local anesthesia may include temporary numbness, tingling sensations, and potential allergic reaction. You may feel some pain in your muscle when the tissue is removed. After the anesthetic wears off, the biopsy site may be mildly tender for 1-3 days. It is likely that the incision site will scar. The scar from the muscle biopsy will be about 1/4 inch long. There is a very small chance that the scars from the biopsy sites may become thick (hypertrophy).
10. Reduced daily eating period (from $\geq$ 12 hours per day to 8-10 hours per day): hunger, fatigue, and hypoglycemia
11. Fasting (for at least 12 hours prior to study visit) for muscle biopsies and venipuncture: hunger, fatigue, and hypoglycemia
12. 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.
13. 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, and/or reapplication of the continuous glucose monitor and/or actigraphy watch if it fell off or did not record data.
14. 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 as the patient identifier on study documents and samples. This document will be kept in a secure location in a locked cabinet. Only the PI and authorized research team members will have access to the cabinet.

15. RISK MANAGEMENT PROCEDURES AND ADEQUACY OF RESOURCES

Strict sterile technique will be observed during venipuncture and muscle biopsy. 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/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. Blood drawing will be minimized to no greater than 200 ml per patient. Subjects will be thoroughly informed of 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. Taub.

Monitoring procedures

Dr. Taub 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. Taub 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

a. Blood draw
    i. Hematoma
    ii. Arterial puncture
    iii. Pain
    iv. Nerve damage
    v. Re-bleeding
    vi. Allergy
    vii. Phlebitis
    viii. Vasovagal reaction
    ix. Anxiety/fear

11. Wrist-worn actigraphy device:
    i. Skin irritation.
12. Continuous glucose monitor
    i. Redness at the sensor insertion site
    ii. Skin irritation (erythema/edema)
    iii. Local infection, inflammation, pain or discomfort
    iv. Bleeding at the glucose sensor insertion site
    v. Bruising, itching, scarring or skin discoloration, hematoma, tape irritation, and sensor or needle fracture during insertion, wear or removal.
13. DXA Scan
    i. Low dose radiation
14. Muscle Biopsy
    i. Pain
    ii. Bleeding
    iii. Bruising
    iv. Infection
    v. Anxiety/fear
    vi. Vasovagal Reaction
15. Reduced daily feeding period (from ≥ 14 hours per day to 8-10 hours per day)
    i. Hunger
    ii. Fatigue
    iii. Hypoglycemia
16. Inability to start lipid-lowering, anti-hypertensive, and anti-diabetics medications or undergo dose adjustments of these medications during the study period
    i. 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).
17. Magnetic resonance imaging/Magnetic resonance elastography
    i. Injury/complications related to presence of certain implanted devices or metallic objects which increase the risks associated with MRI. Such devices include certain intracranial aneurysm clips, cochlear implants, certain prosthetic devices and pacemakers. Injuries/complications include device movement, burns, and device malfunction.
    ii. Discomfort due to the vibrating paddle of the MRE.

AE Management

18. 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. Taub, who is a cardiologist, will be overseeing the procedure to check for the patient's vital signs. Dr. Taub'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 (ACTRI) is equipped with trained medical staff (physicians and nurses) who can respond quickly. Should an event occur, Dr. Taub 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. Taub will monitor the patient until the problem has been resolved or has stabilized.
19. 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).
20. Muscle Biopsy
    a. Prior to enrollment into the study, the patient will be educated on the risks of getting a biopsy from their leg muscle. Dr. Taub or Dr. Wilkinson 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 muscle biopsy will be thoroughly sanitized with an alcohol wipe prior to the incision with scalpel and the needle stick. Before the procedure is performed an area of skin and the deeper tissues on the outer front portion of either one of the patient's lower thighs will be numbed using anesthetic (xylocaine) and then the small incision will be made with the scalpel. The special biopsy needle will be inserted through the incision and into the thigh muscle with extreme care several times and a small amount of tissue will be removed (about the size of a small pea). Deep pressure will be applied on the area for 20 minutes to minimize the risk of bleeding and then a steri-strip tape will be used to close the incision. Dr. Taub or Dr. Wilkinson, who are cardiologists, will perform the procedure. Dr. Taub has performed over 200 muscle biopsies with no complication. Dr. Taub'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 (ACTRI) is equipped with trained medical staff (physicians and nurses) who can respond quickly. Should an event occur, Dr. Taub 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. Taub will monitor the patient until the problem has been resolved or has stabilized.
21. 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 protocol established by the device manufacturer: 1) Wash hands with soap and water, 2) Choose sensor insertion site on upper arm, 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, 6) Once sensory filament is inserted into the subcutaneous layer, an adhesive product may or may not be used. 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 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 (ACTRI) is equipped with trained medical staff (physicians and nurses) who can respond quickly. Should an event occur, Dr. Taub 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. Taub will monitor the patient until the problem has been resolved or has stabilized.

22. DXA Scan
    a. Prior to enrollment into the study, the patient will be educated on the risks of obtaining a DXA 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 DXA 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. Taub 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. Taub will monitor the patient until the problem has been resolved or has stabilized.
23. Reduced daily feeding period (from $\geq$ 14 hours per day to 8-10 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 or type II diabetes on medical therapy. 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. Taub will monitor the patient until the problem has been resolved or has stabilized.
24. 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. Taub will monitor the patient until the problem has been resolved or has stabilized.

25. Magnetic resonance imaging

a. Prior to enrollment in the study, the patient will be carefully screening for the presence of any implanted devices or internal metallic objects. If such devices and objects are not compatible with MRI (MR Unsafe), the patient will be excluded from the study. MR Conditional devices will be reviewed on a case-by-case basis by the Principal Investigator, and patients with such devices may be excluded from the study.

b. Patients will be screened for a history of claustrophobia and/or anxiety related to prior MRI imaging, or any history of intolerance to MRI imaging for any reason. Patients will be counseled and prepared for the MRI study, including the duration of study, steps taken during the study, and will be informed that the MRI procedure may be associated with claustrophobia, discomfort due to loud noise, and/or anxiety in some patients. Patients may discontinue the study or decline to participate in the MRI portion of the study due to concerns related to claustrophobia and/or anxiety. MRI compatible hearing protection will be provided as part of routine care during MRI imaging.

c. In the event that the vibrating paddle used for the MRE is uncomfortable to a subject, the subject will have been instructed to tell the MR technologist if the vibrations become uncomfortable, and the MRE part of the examination will be discontinued.

d. 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. Taub 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 electronic forms and will be identified with the study's subject ID. 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

Patient privacy and confidentiality will be addressed by adhering to data securing measures. These measures include use of a password protected, secure, internet-based database management program.
All study forms including consents, HIPAAs, and case report forms (CRFs), which will include elements from the present and past history, patient demographics, including name, medical record number, age, current medications and lab draws/results, will be stored in in a locked cabinet in Dr. Taub's research office in the ACTRI building. When not in use CRFs will be stored in a locked cabinet or office within a limited-access facility. Access to the CRFs will be given to study staff only. Information collected by study staff for study purposes will be kept confidential and will not be shared with anyone, including study participants and sponsors, outside of the study staff or IRB. To minimize the potential loss of confidentiality and privacy, patients will be assigned a unique number as their subject identifier code. The unique subject code will be used to label all study documents. This information will only be available to study personnel and will be secured in the same manner mentioned above.

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. Taub's research office in the ACTRI building. Samples will also be sent to Dr. Panda's lab at the Salk Institute for storage and analysis.

The research team will uphold the standards of HIPAA and patient privacy as allowed by law. The following Personal Health Information will be collected for research purposes: name, date of birth, medical record number, phone number, email, address. The following will also be collected through the mCC app: travel plans, timestamp of food/beverage intake and geographic location data.

mCC app data: Daily nutrient dates will be collected from each subject. The user's sleep and activity data may be collected by sleep log questions in the app and by passive monitoring of accelerometer in the phone. When the subject starts eating they will launch the app on the phone and take a photograph of the item(s) consumed. Beverages and water are also considered as food for the purposes of this study. The app includes an optional provision to append a brief textual message to accompany the photograph, in case the subject intends to add any remarks regarding the photograph that may not be obvious from the photograph itself (i.e. Brand names, size, etc.).

For any reason, if the subject does not wish to record a photograph of the food item(s), he or she has the choice of:

- choosing from a list of foods commonly consumed and the approximate time that food was consumed by the subject
- typing out the details of what he or she ate and the time that food was consumed

These two options can be also be utilized to retroactively log the food consumed in case the subject forgets to take a photograph at any mealtime.

Upon pressing send, the photograph (which is sent as a JPEG file) and/or the textual content (which is sent as a text file) is transmitted to a cloud server. The URL for this server is Circadianstudy.org and the name of the file that is transmitted to us is of the format:

<unique id>_<date>_<time>.<file extension (jpg or txt)>
e.g. 2b6f0cc904d137be2e17_2011-08-15_11-08-36.jpg

The files received in the server will be regularly monitored by the investigators to ensure the smooth progress of the study. In particular, if a user forgets to log the data for two to three standard meal times, he or she will be sent a push notification reminder automatically by the server.

User input: The overall layout of the data input, storage and analyses is shown in Fig 3. The default and recommended mode of collecting data will be taking a picture of any food or beverage an individual ingests. As soon as the app is activated, it will seek the instantaneous geolocation (longitude, latitude). The individual can take a picture of the food, can choose to use the picture or retake and makes sure there is no personally identifiable item in the picture (ID card, another person's face at a conference table, etc). Once s/he is satisfied with the quality of the picture, the individual will have the option of adding a short annotation for the item. Such optional text entry will describe the food, (item name, decaf or sugar free, portion size, whether the picture represents leftover from the previous meal etc.). Every textual entry will populate an individualized database of foods or beverages consumed. Thus, the app will learn the subject's entries, so that over time, it can auto-suggest these annotations. The first time the app is launched it creates and stores a 20 character alphanumeric identifier for the device, e.g. Bw2iAm67juEglgN1qK8t. This identifier is unique and permanent for the duration of the study, stored on the device as well as the server and serves the purpose of uniquely but anonymously keeping track of multiple users.

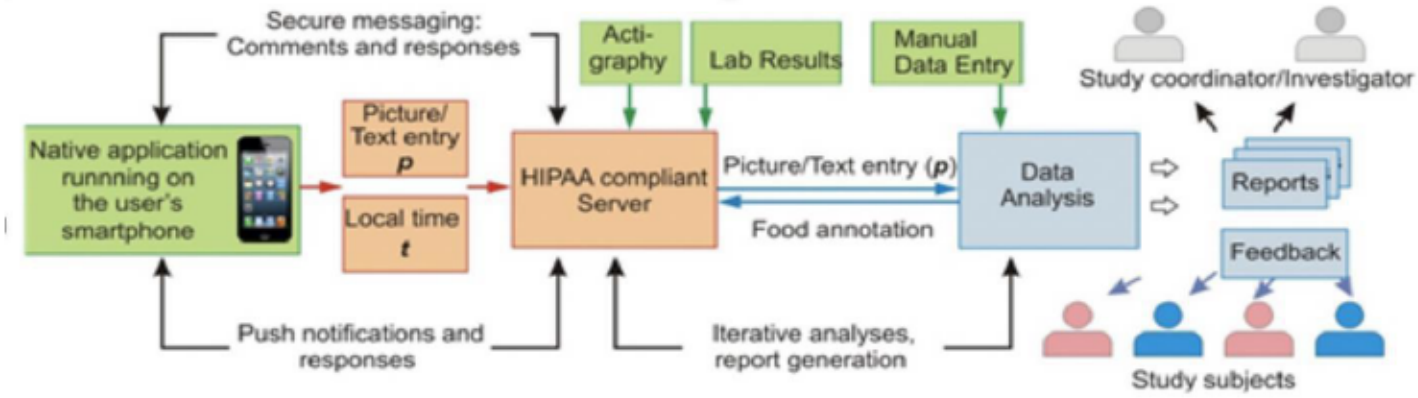

Fig 3. Overall layout of the data acquisition, storage, and analyses plan.

The second option is to scan a barcode or a QR code of a food/beverage/medication item. Scanning the code simplifies downstream data annotation. Food manufacturers are required to label or offer detailed nutritional information. The app's barcode scanner is used to retrieve the manufacturer's data during the subsequent annotation step.

The third option is to manually type (text entry) the food item. This feature will be mostly used for situations where taking a picture of the food is not socially acceptable or if the subject forgets to record an event. As soon as the individual fills out the text field, a time wheel will automatically pop up asking whether the event is current or happened in the past. The current version of the app does not have a barcode scanner and the text and camera functions are in different tabs. In the proposed version all three modes will be integrated into one screen and the end-user can seamlessly switch between these data gathering modes.

After the picture/barcode/text is entered, the user will choose whether the item is "water", "beverage", "Food" or "Medication/supplements". This simple act will sort the items into four major classes of ingested items and greatly simplify downstream annotation. At the beginning of the study, the participants will be encouraged to

annotate, at least once, any medication or supplements they are taking in the text field of a picture. This data-sorting at user-end is a new feature over the current prototype.

Data transmission: As the user annotates the picture, the app will name the picture or text file with a unique identifier. The identifier will begin with the user ID followed by the timestamp. Network congestion can sometimes slow down the data transmission, cause frustration and reduce compliance. In the current app this varies from less than a second to a minute, during which the screen freezes. Therefore, the picture/text along with the time stamp and geo-location will be stored in a temporary onboard cache while the network functionality is getting restored, and once the app is able to connect to the internet, it can transmit the data to the server. All picture files are reduced in size by a factor of 10 or 15 to make the final image file size <300kb. At this size the pictures have enough resolution for identifying food items, while minimizing the data transmission time to one-tenth. Each outgoing data package from the smartphone will have three pieces of information; **(1)** a picture or textual entry <subject identifier>_<sorting tag>_<date>_<time> e.g. Bw2iAm67juEglgN1qK8t_f_2011-11-11_18-12-58.jpg **(2)** annotation text e.g. Bw2iAm67juEglgN1qK8t_2011-11-11_18-12-58.dsc and **(3)** Location tag e.g. Bw2iAm67juEglgN1qK8t_2011-11-11_18-12-58.loc

Personalized push notification: Preliminary data has indicated that personalized push notifications (which appear as a badge resembling that for text messages) few times a week improves compliance with data collection. Push notifications are widely employed by most apps and most users are well-versed with their usage and utility. The geo-location tag will be used to calculate the local sunrise-sunset time and the reported events in the past 2 days will be used to compute the time period when the subject is likely active. A simple push notification will be automatically sent to the individual asking "Did you eat or drink anything in the last 30 min?" with a two option "Yes/No" response. To account for the possibility that there might be a time lag between when the push notification is sent and when the user notices it on the phone, the time interval used for generating the yes/no question will encompass the 30 min period prior to the user noticing it (and not when the push notification is sent by the server). The subject's response (Yes or No) along with the presented question and the time at which it was answered will be transmitted back to the server where it will be compared with the timestamp of data packages received within the last 30 min (this time interval will need to be optimized in trials). Such push notification and response gathering serve two purposes. First, push notification improves participants' engagement with the app and improves reporting over extended period of time. Second, it helps obtain an error estimate for the individual participants. Concordance between an event in the given period and response is expected, but discordance will be taken into consideration for error estimates. There are two major types of errors: when a person recorded an event, but answered no and when a person did not record an event and answered yes. In the second case, the subject will be prompted to text the missed event.

Sleep log: Although sleep and physical activity will be passively monitored with the smartphone's accelerometer, a tab in the app will encourage users to input their self-reported sleep parameters as has been extensively used in many peer reviewed studies. Throughout the study participants will take the Pittsburgh Sleep Quality Index (PSQI) questionnaire. PSQI's administered in one month interval differentiates transient sleep problems from persistent problem and has a test-retest reliability of >80%. Combination of PSQI, periodic post-sleep questionnaire, and accelerometer will be powerful tool to evaluate sleep and activity. During the course of the study the subject will be encouraged to go to the sleep log tab before going to bed where s/he will simply push the button – "going to bed" and every morning the app will automatically switch to the sleep window, so that the participant can push "woke up" and rate his/her sleep on a 4 step sliding scale from "Very good" to "Very bad" (same as Q6 of PSQI). If the subject reports bad sleep, Question 5 of PSQI asking probable cause of bad sleep will appear. During the day, the subject will also be asked to record any nap by clicking two buttons "going to nap", "finished napping". These answers are sent to the server along with geo-tag and timestamp. Such engagement with the subject serves two purposes – to get a better estimate of

person's self-reported sleep and a better estimate of the geo-location of the subject's "home". We anticipate in the next several months the smartphones will improve their accelerometer function, so that activity and sleep can be passively measured.

Database architecture: The files and databases will be hosted on a commercial cloud computing platform: Amazon Web Services (AWS). AWS provides EC2 (Elastic Compute Cloud), a platform for HIPAA compliant on-demand computational capacity via virtual machines called "instances" that range from low capacity to very high capacity in terms of CPUs, RAM and storage. Further, the computational capacity can be rapidly and conveniently scaled up or down as needed. We will instantiate 2 large capacity Linux servers (main server and backup) with load balancing to capture the data that the smartphone users transmit. The data will be stored on AWS S3 (Simple Storage Service). These two servers will remain online 24h a day, 7 days a week. A third EC2 server will regularly retrieve incremental data from the main server, run processing and analytics jobs, create and dispatch mTurk tasks and provide visualization of the data to the study coordinators. We will use the relational database MySQL to store data and PHP (programming language) to interact with this database. The following tables will be maintained in one database: (1) table to store the file names for the data package triplet: picture/text, description as entered by the user, geo-location (2) alphanumeric identifiers generated for each user at the time of activation of the app, the user's study start date and his/her Apple push notification ID used to send push notifications (3) the times at which push notifications were triggered by the server and (4) the user responses to those, and (5) the responses to the sleep and behavioral questionnaires. A second database will record the annotations for each image. To maintain data integrity and prevent data loss, all data received from study participants as well as that residing in the MySQL databases will be mirrored on the backup AWS instance.

Image analyses: State-of-the-art image recognition algorithms cannot reliably identify food items, so manual annotation of the food is necessary. Presorting of the food types to water, medication/supplements and food/beverages by the app users will streamline image analyses. From the preliminary study we find up to 30% of events involve water intake. The rest 70% of images were largely calorie-containing food or non-water beverage items. Lastly, a small fraction is nutrient supplements and medications. Crowd sourcing may be used to annotate the collected images to identify the food and beverage items and extract nutrient information. A "gold standard" reference dataset comprise of images obtained from previous participants and annotated by a trained dietitian/nutritionist will be administered to every crowdsourcing annotator. This gold standard dataset will be a reference platform for estimating the reliability of the individual's annotations. Crowdsourcing as a first step has several advantages. The Amazon Mechanical Turk (mTurk) platform enables recruiting as many or as few individuals spread all over the globe for an extremely low cost. Crowdsourcing can be done 24/7 and is cost-effective. Since a large crowd can be mobilized to a given task, thousands of food pictures can be annotated within days or even hours. More than half of the food and beverages consumed by free-living individuals are prepackaged food or prepared meals from a fast-food or normal restaurant. Collecting nutrient information from these food items involves look-up tables from CDC or Nutrient Data Laboratory (http://www.ars.usda.gov/main/site_main.htm?modecode=12-35-45-00). Food is cultural, has rich diversity and food complexity is constantly changing. So, information on complex or food unique to a culture or geographic location is more likely to be annotated faster by first obtaining a provisional annotation by the local "crowd" and subsequent verification by nutritionists. Each food or beverage item will be first named and various parameters will be estimated, including calories, volume, macronutrient content, pre-packaged/prepared or home-cooked, fruits and vegetables, taste modality (sweet, sour, bitter, salty, umami), alcohol content and type (wine, beer, spirit). Water annotation serves two purposes; to estimate the daily fluid intake by individuals and to estimate supplemental intake through bottled water (Vitawater™, Smartwater™ etc). Medications and nutrient supplements are difficult to annotate based on picture alone. So, at the end of the monitoring period, a sampling of medication pictures which cannot be annotated will be sent back to the participants for identification. Although the intervention is based on time of eating, the picture annotation will help evaluate

whether changing the eating pattern indirectly improves nutrition quality and reduces total calories by enabling individuals, for example, to reduce after-dinner snacks, desserts or alcohol.

Geo location: Geo-location of food intake offers insights to the eating pattern, living environment and lifestyle of individuals. Collection and analyses of this information can improve both personalized nutritional and lifestyle recommendation as well as help in public policy formulation. As the geographic information system maintained in local and federal government becomes digitized, annotations for each geolocation can eventually be automated in the future. However, for the current project, we will rely on crowdsourcing for annotating each geolocation. Each geolocation will be mapped using a web-based mapping program. The median resolution of geo-location (latitude and longitude coordinates) in our pilot study has been 30 ft. Geolocations collected at evenings and nights and those associated with response to sleep questions will be clustered to find the subject's "home" as the location (within ~300 ft. resolution) with the maximum number of night time events. Similarly, the cluster of weekday daytime geolocations (within ~300 ft.) resolution will be annotated to find approximate "work" location. The remaining geolocations represent other locations including transit, restaurants, other residential place (friend/family) places of socialization, gym, parks etc. Some subjects may not have a well-defined "work" address. Finally, the geo-tag classifier will be linked to the food annotation. As a result we can classify whether a food was consumed at home, work or other location and accordingly quantify various parameters of food with location.

Daily Activity Rhythms/Sleep Quality

Although several smartphones have inbuilt accelerometer and growing number of people use wrist worn commercial accelerometers, heterogeneity in the type of accelerometers and communication method between the app and the meter may not allow us to collect activity data from all participants. When this communication is feasible through APIs and SDKs, we will collect activity/sleep data from the device.

Optional data fields:

The app will have optional data fields to record mood, hunger level, alertness/sleepiness level, blood pressure, heart rate, bowel movements, pulse oximetry, temperature, ketone bodies (urine and or blood sample), and results from blood samples including: blood glucose, total cholesterol, low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), triglycerides, Hemoglobin A1c, Fibrinogen, C-Reactive Protein, and Homocysteine. A few controlled on a small size study population have reported insufficient sleep and erratic daily eating pattern can increase risk for metabolic diseases or worsen prognosis of these diseases. By collecting these additional parameters, we will be able to test in a large population if erratic eating pattern and insufficient sleep correlate with poor health status.

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 metabolic syndrome. The potential benefit to the individual to those randomized to the TRE group is that by engaging in TRE, subject may see overall improvement in cardiometabolic risk factors and decreases and biomarkers, which may improve subject's overall health and reduce risk for cardiovascular disease. There may also be improvements in sleep quality and mood. There may be no benefit to those randomized into the standard of care group.

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 patients with metabolic syndrome, including weight loss.

19. EXPENSE TO PARTICIPANT

There will be no expense to participants.

20. COMPENSATION FOR PARTICIPATION

Patients will be compensated with up to $400 for fully participating in the study. Parking for the study visits will be paid for by the research team. We do not provide compensation for cell phones, cell phone data, or Wi-Fi used for the app.

Participants will receive the following compensation per visit:
Visit 0 or 1 - $50
Visit 2 - $150
Visit 3 - $50
Visit 4 - $150

21. PRIVILEGES/CERTIFICATIONS/LICENSES AND RESEARCH TEAM RESPONSIBILITIES

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

Satchidananda Panda, PhD (Co-Investigator) is a researcher at the Salk Institute for Biological Studies. He will be responsible for the following aspects of the project design, data analysis, manuscript writing, and mCC app oversight.

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

Hemal Patel, MD, PhD (Co-Investigator) is a Professor of Medicine at UCSD. He will be responsible for the following: data analysis and manuscript writing.

Michael Wilkinson, MD (Co-Investigator) is an Assistant Professor of Medicine at UCSD and is board certified in internal medicine, cardiovascular diseases, and clinical lipidology. He will assist with the following: project design, patient recruitment, muscle biopsies, data analysis, and manuscript writing.

Jia Shen, MD (Co-Investigator) is an Assistant Professor of Medicine at UCSD and is a board-certified cardiologist. She will assist with the following: patient recruitment, data analysis and manuscript writing.

Iwona Swiatkiewicz, MD, PhD (Co-Investigator) is an Associate Professor of Medicine at Nicolaus Copernicus University and a board-certified cardiologist. She is a visiting scholar who will assist with the following: patient recruitment, data analysis, and manuscript writing.

Adena Zadourian, BS (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, and manuscript writing.

Hannah Lo, BS (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.

Cameron Ormiston, Nancy Tomka, Aryana Pazargadi, Ashley Rosander, Emily Ross, Nikko Gutierrez, Briana Sanchez, Juancarlos Cancilla, Sofie Maisel, Sneha Panda, Shelley Mitra, David Van, Christopher Van, Erika Padilla, Parnia Abolhassan-Choubdar, Justina Nguyen, Brian Khov, and Victoria Green (Research Assistant) have undergone proper training and obtained the certificates necessary to work with human subjects and they will manage and assist with the following: patient recruitment, patient scheduling, IRB submissions, administrative and technical support, and data collection and analysis.

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

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24. BIOLOGICAL MATERIALS TRANSFER AGREEMENT
MTA is under review
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.

2. Original Protocol

UCSD Human Research Protections Program
New Biomedical Application
RESEARCH PLAN

Instructions for completing the Research Plan are available on the HRPP website.
The headings on this set of instructions correspond to the headings of the Research Plan.
General Instructions: Enter a response for all topic headings.
Enter "Not Applicable" rather than leaving an item blank if the item does not apply to this project.
Version date: 9/30/2013

1. PROJECT TITLE

Influence of time-restricted eating (TRE) on circadian regulation of glucose homeostasis and mitochondrial function

2. PRINCIPAL INVESTIGATOR

Pam R. Taub, 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

5 years

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

Circadian rhythms optimize nutrient homeostasis by orchestrating catabolic and anabolic metabolism to appropriate times of the 24 hour (h) day. Chronic circadian rhythm disruption predisposes individuals to metabolic diseases including obesity and type 2 diabetes. Conversely, maintaining a daily rhythm of feeding and fasting cycles sustains a robust circadian rhythm which improves cellular bioenergetics and results in improved metabolism. Time-restricted eating (TRE) is a specific feeding-fasting pattern in which feeding is restricted to 8-12 h a day.
In a randomized controlled trial, we intend to measure the health impact of TRE in patients with metabolic syndrome (with three or more of the following criteria: increased waist circumference, abnormal cholesterol levels, elevated blood pressure, or elevated blood sugar), who habitually eat for more than 14 h every day. 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 evaluate the impact of TRE on blood glucose levels, metabolic biomarkers, body composition, and mitochondrial function. These assessments will be made at baseline and at the end of the 12-week intervention period. Food/drink intake, activity, and sleep will be monitored with the smartphone myCircadianClock application ("mCC app") throughout the study.

6. SPECIFIC AIMS

We hypothesize that imposing consistent feeding-fasting cycles will restore the equilibrium between catabolic and anabolic processes, which will improve glucose homeostasis and cardiometabolic biomarkers, decrease abdominal fat and enhance mitochondrial function. We will test this hypothesis with the following specific aims:

Specific Aim 1 – Evaluate the impact of TRE on glucose homeostasis.
Hypothesis 1: The TRE group will have significantly lower mean blood glucose levels compared to the control group. The primary endpoint will be the change in glucose levels assessed via continuous glucose monitors (CGM).

Specific Aim 2 – Assess changes in metabolic biomarkers.
Hypothesis 2: TRE induced decreases in blood glucose levels will be accompanied by improvements in biomarkers such as LDL particle number via NMR lipoprofile, hs-CRP, and HbA1c, which reflect cardiometabolic stress.

Specific Aim 3- Assess the impact in body composition.
Hypothesis 3: The TRE group will have significantly lower abdominal fat mass compare to the control group at the end of the study as assessed by DXA.

Exploratory Aim 1 – Elucidate the effects of TRE on changes in mitochondrial structure and gene expression in skeletal muscle.
Impaired glucose homeostasis can result from insulin resistance, compromised mitochondrial function, adiposity, dyslipidemia, and inflammation. To elucidate the underlying mechanisms, skeletal muscle biopsies will be utilized to analyze 1) alterations in mitochondrial structure/function and 2) changes in gene expression of candidate target genes triggered by TRE.

7. BACKGROUND AND SIGNIFICANCE

Metabolic syndrome. In the United States, >34% of adults meet criteria for metabolic syndrome, and prevalence increases with age. Metabolic syndrome is characterized by the presence of multiple risk factors for cardiovascular disease and diabetes (see criteria outlined in Table 1) [1, 2]. Compared to unaffected individuals, the presence of metabolic syndrome doubles the risk of developing cardiovascular disease over the next 5 to 10 years, and it is associated with a 5-fold increase in the risk of type-II diabetes mellitus (T2DM) [1]. In patients with T2DM, cardiovascular disease is the leading cause of death.

While lifestyle modification is currently the first line of treatment for metabolic syndrome, it is usually not very effective as the patients' condition often deteriorates. The diet currently recommended for patients with metabolic disease is not ideal [3]. Second, lifestyle programs administered in a clinical setting rely heavily on weight loss through calorie-reduced diets, which is an approach that is effective at reducing body weight and improving cardio-metabolic risk factors in the short- to medium-term, but often fails in the long-term given that many individuals fail to adhere to low-calorie diets for more than 6 months [4]. Moreover, for patients with clinically normal body weight and metabolic syndrome, there is very little room for weight loss. Thus, there is an unmet clinical need to identify better approaches for long-term body weight control and lifestyle modifications that affect glucose homeostasis-independent of changes in adiposity. *Therefore, novel mechanisms that can act independently of weight loss or altered nutritional content are needed.*

Metabolic diseases: Disruption in metabolic homeostasis underlies metabolic diseases. Nutrient homeostasis (e.g. of glucose, lipid, cholesterol and amino acid metabolism) is achieved by concerted action of (a) sensors that detect the cellular or organismal nutrient demand and nutrient availability, which in turn instruct the regulators and enzymes that mediate (b) anabolic or (c) catabolic pathways [5-7]. Several intermediate metabolites serve as co-factors, ligands, scavengers, electron/proton/carbon/active group carriers (e.g. Heme, sterols, bile acids, nicotinamide adenine dinucleotide (NAD+)) for anabolic or catabolic reactions. Research on metabolic homeostasis has led to the identification of these sensors, regulators, enzymes, and intermediate metabolites which upon hyper- or hypo-activation disrupts homeostasis thus leading to metabolic diseases. Overall,

homeostasis is maintained by equilibrium between anabolic and catabolic pathways. *One mechanism contributing to this equilibrium is the circadian rhythm, such that anabolic and catabolic pathways are active at different times of the 24 h day [8].*

Circadian rhythm and daily eating pattern regulate daily rhythm in metabolism. Cell autonomous circadian oscillators composed of transcriptional activators (CLOCK, BMAL1, NPAS2, ROR) and repressors (PER, CRY, REV-ERB) (henceforth called "clock components") are present in almost every organ [9]. The circadian oscillator in the hypothalamic suprachiasmatic nucleus (SCN) acts a master clock that generates daily rhythm in physiology and behavior such as activity and rest [10]. To adapt to this daily rhythm in activity-rest the circadian clock in different organs orchestrate daily rhythms in neuroendocrine factors that sense and signal energy states in different organs, rhythms in hunger and satiety hormones, and rhythms in function of hundreds of genes involved in catabolic and anabolic metabolism in organs such as liver, muscle, and adipose tissues [8, 11]. As a result, the circadian clock drives a daily cycle of feeding and fasting and orchestrates mechanisms to activate anabolic pathways to store nutrients during period of feeding and activate catabolic pathways to utilize the stored nutrients during period of fasting. Cellular metabolism produces reactive oxygen species (ROS) and molecular damage. Accordingly, circadian clocks also support a daily rhythm in cellular repair mechanisms including autophagy, mitochondrial fission-fusion cycle and ROS scavenging molecules [12-14]. Such rhythms in repair mechanisms sustain structural and functional integrity of organelles such as mitochondria [15].

To adapt to seasonal changes in day length, light entrains the SCN circadian clock to day:night cycle, which in turn adjusts the circadian clock in peripheral organs. However, the peripheral circadian clocks in metabolic organs including the liver responds to eating time, so that an abrupt change in eating time can reprogram the phase of the circadian clock and its output genes in the liver without affecting the SCN clock [16-18]. This "food-entrainment" of the peripheral clock may allow for rapidly adjusting the periods of anabolic and catabolic metabolism to the new timing of food availability, so that homeostasis is maintained.

Such acute effect of food on circadian rhythm forms the basis for understanding how daily cycles of feeding and fasting affect circadian gene expression and metabolic homeostasis. Systematic analyses of circadian gene expression in the mouse liver have revealed a dominant role of eating pattern on daily rhythms in gene expression. In the liver of fasted mice, only ~350 transcripts including the clock components and their direct output targets show daily oscillations. The normal pattern in mice when fed a normal chow *ad libitum*, is the consumption of the *majority* of calories at night when they are normally awake and their liver shows rhythmic expression of ~3,000 transcripts. Allowing mice to eat all of their food during their normal wakeful time only without altering caloric intake increases the number of rhythmic transcripts to ~5,000, which has a beneficial effect on metabolism. Conversely, (a) when mice eat randomly throughout the day and night, so that they consume more food when they are supposed to sleep, or (b) in mutant mice that lack clock components, daily rhythms in gene expression are dampened or abolished [8, 11]. These animals gradually succumb to metabolic diseases. In summary, these studies concluded that a daily rhythm in feeding and fasting supports a robust rhythm in numerous mRNAs and proteins, which regulate metabolic processes such as gluconeogenesis, glycolysis, protein synthesis, cholesterol synthesis, lipid synthesis or oxidation and mitochondria function.

Why are mRNA or protein rhythms beneficial? Majority of the genes that show daily rhythms in expression or activity play important roles in metabolism. Some of these key metabolic regulators include (cAMP-Response element-binding protein (CREB), activating transcription factor 6 (ATF6), mechanistic target of rapamycin (mTOR), forkhead box protein (FoxO), sirtuin proteins (SIRTs), sterol regulatory element- binding protein (SREBP), and (peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) [19]. Furthermore, many of the clock components also directly regulate the expression of genes involved in nutrient metabolism [14]. *Daily rhythm in the function of these proteins with their peak activity timed to periods of fasting or feeding ensures the metabolic pathways they control are active only for a few hours every day when needed. For example, CREB mediated gluconeogenesis is active only for a few hours during fasting or mTOR pathways is active only during feeding. This leads to metabolic efficiency and ensures optimal function that supports normal health. Constitutive (constant) activation or constant suppression of these metabolic regulators is often found in metabolic diseases including diabetes, obesity, and fatty liver disease. Circadian clock mutant mice and animal*

models of chronic shiftwork show dampened gene expression rhythms and these animals are predisposed to metabolic diseases. Therefore, lifestyle or pharmacological interventions that improve daily rhythms in gene expression and function are expected to improve metabolic health.

Circadian disruption increases risks for metabolic syndrome. Circadian rhythm disruption can be due to genetics (rare among humans) or lifestyle. Lifestyle induced circadian disruption is mostly due to (a) shiftwork - working (and staying awake) during habitual sleep time and trying to sleep during habitual wakeful hours, and (b) sleep deprivation – increase in the length of activity period. Shift workers, who account for >20% of the workforce, typically return to their daytime social life during off-days, and experience the internal drive for sleep and wakefulness. As the phase of circadian oscillator does not abruptly change in response to abrupt change in light:dark cycle as occurs in shiftwork, repeated resetting cues disrupt the normal circadian rhythm. A large body of epidemiologic and clinical evidence has indicated that shift work or sleep disturbances is associated with an increased risk of T2DM [8, 20-27]. Shift workers also experience erratic eating patterns. Erratic eating patterns, such as late night caloric intake, increase the risk of developing coronary heart disease by as much as 55%, even after controlling for diet and lifestyle [28]. Modeling shiftwork in animals disrupts the normal molecular circadian clock and predisposes them metabolic diseases [29]. Even in non-shiftwork animal models, erratic eating pattern causes circadian rhythm disruption and metabolic diseases [30, 31].

Aging animals and humans also show dampening of daily rhythms at neuroendocrine and physiological parameters [32] and the risk for metabolic diseases increases with age. Finally, in model organisms, genetic perturbation of the circadian clock abolishes their daily rhythm in activity and feeding and increases the incidence and severity of metabolic diseases [33-36].

In summary, these powerful observations in both animal models and humans support the premise that chronic circadian rhythm disruption can increase the risk for metabolic diseases. However, the underlying mechanism in most studies is confounded by the fact that chronic circadian disruption often accompanies sleep restriction, consumption of energy dense diets and/or erratic eating pattern. The proposed work will make key contribution to this knowledge gap by addressing the effect of altering duration of eating pattern on circadian regulation of metabolism.

Time-restricted eating: Time restricted eating (TRE) for 8-12 h - without reducing caloric intake relative to ad lib fed (ALF) controls – is an experimental feeding regimen that is effective in testing the role of daily feeding-fasting rhythm in circadian rhythms and in metabolic homeostasis. As mentioned earlier, ad libitum access to energy dense high fat diet dampens the molecular circadian oscillator [30], dampens the daily feeding rhythm [37], and is associated with obesity and metabolic diseases. Conversely, TRE of diet rich in carbohydrate, fat, fructose, or sucrose without reduction of caloric intake increases the robustness of circadian clock and can prevent or even reverse cardiometabolic diseases found in animals fed an identical diet ad libitum [38]. TRE exerts pleiotropic beneficial effects on multiple organ systems of mice (liver, muscle, white adipose tissue, brown adipose tissue, gut, and brain) [37-41] and insects (muscle, heart, brain) [42]. Mutant animals lacking circadian clock components do not exhibit the benefits of TRE, thus suggesting TRE acts through the circadian clock [42].

The most profound and well described effect of circadian rhythm disruption on metabolism in both rodents and humans is in glucose homeostasis. In healthy adults, glucose tolerance and insulin sensitivity exhibit diurnal rhythms [43-45]. **Circadian disruption, even for a few days, can cause insulin resistance or glucose intolerance [26, 27]**. In animals fed an obesogenic or high glycemic diet, TRE can restore normal glucose regulation [38]. The underlying molecule mechanisms of TRE restoring glucose homeostasis described in rodent relates to restoring daily rhythms in mTOR and AMPK function. The anabolic mTOR pathway downstream of insulin is mostly active during the fed state, while the glucagon and AMPK-driven pathways leading to gluconeogenesis and fatty acid oxidation pathways are only active for a few hours during the habitual fasted state. Diet induced obesity or frequent eating causes both these pathways to be continuously active throughout the 24 h, which abolishes the circadian rhythm [37]. TRE restores normal circadian clock function and the daily rhythms in mTOR and AMPK function. In concert, TRE also restores normal levels and/or normal daily rhythms several mRNAs, proteins, and metabolites that are implicated in metabolic homeostasis of lipids, cholesterol, redox and mitochondria function [37].

In summary, these observations suggest that erratic eating pattern or extended period of eating disrupts circadian rhythms and predisposes to metabolic diseases and TRE in animals restores these rhythms and prevents or reverses the diseases. This hypothesis will be tested in the proposed study by subjecting adults with metabolic syndrome who habitually eat over a >14 h duration to a 10 h eating window (with a 14 hour fasting period) and examining whether TRE improves glucose homeostasis.

TRE and mitochondria function. Mitochondria play a central role in nutrient homeostasis, and impaired. mitochondria function and structure are common hallmarks of metabolic diseases in both humans and animals [46]. Conversely, many pharmacological and lifestyle interventions that improve mitochondria functions and structural integrity also alleviate metabolic diseases [47]. The circadian clock regulates several functions of mitochondria, and maintains its structural integrity [48]. Genetic or high fat diet induced circadian rhythm disruption compromises mitochondria function as well as structure [36, 48, 49] , which parallels development of metabolic diseases. The beneficial impact of TRE on mitochondria function in animals has been observed from molecular signatures to whole organism's fitness: gene expression, metabolites, mitochondria structure, mitochondria function, and physical activity. TRE selectively increases expression of PGC1α by 280%, a 2.8 fold increase [37, 38]; PGC1α is the master regulator of mitochondria biogenesis and of several mitochondrial proteins that function in the central metabolic pathways of mitochondria-such changes are observed at both the RNA and protein levels. Metabolomics analyses have revealed TRE increases the net metabolic flux through the mitochondrial Tricarboxylic Acid Cycle (TCA) and fatty acid oxidation [37, 38], which contributes to improved glucose homeostasis in these animals. TRE effects are conserved across species and are observed in Drosophila, where TRE led to reduction in expression of mitochondrial electron transport chain (ETC) components [42]. Reduction in ETC gene expression likely reduces the production of reactive oxygen species (ROS) and preserves mitochondrial structural integrity. Quantitative microscopy techniques have revealed mitochondrial volume and overall structure improves with TRE in rodents [37]. The improvement in mitochondria function is pervasive as it is observed in multiple organs in mice and Drosophila. These biochemical and ultrastructural improvement in mitochondrial function translates to improved physiology; TRE improves muscle function as measured by endurance in mice, flight ability and cardiac contractility in Drosophila [37, 38, 42].

While these animal studies clearly indicate TRE improves mitochondria function and structure in both liver and muscle tissues, whether circadian rhythm restoration through TRE can restore mitochondria function and structure in humans has never been explored. This proposal will directly test this hypothesis by examining gene expression, protein levels and structure of muscle mitochondria from subjects undergoing TRE.

8. PROGRESS REPORT

None.

9. RESEARCH DESIGN AND METHODS

Overall Study Design. Participants will be recruited from the cardiovascular and internal medicine clinics at UCSD and through a database search with EPIC, and we will register with www.clinicaltrials.gov. We plan to enroll 144 participants, age 18-75 years who meet criteria (refer to Section 10). Participants must have a self-reported dietary intake of ≥14 hours per day, regular daytime schedule of activity (no shift workers), and self-reported habitual sleep duration of >6.5 h. prior to study enrollment. The study will consist of 1 week of screening, 1 week of baseline , and then they will be randomized into one of two groups (standard of care or standard of care with TRE) for 12 weeks (14 weeks total). They will utilize the mCC app for the entire 14 weeks. Testing will include blood draw, anthropometric data, vitals, wrist-worn actigraphy device, muscle biopsy, and DXA scan before and after the intervention period.

Screening. Participants meeting eligibility criterial will be identified based on a database search through the UCSD patient medical record (EPIC), ResearchMatch, physician referrals, and flyers. A detailed phone interview will be conducted to further determine if the subject meets the inclusion and exclusion criteria. The Beck Depression Inventory (BDI) will be used to screen for depression and the Epworth Sleepiness Scale (ESS) will be used to exclude for sleep apnea. If participants pass this phase of screening, they then will be asked to come to the ACTRI to be enrolled and consented into the study at Visit 1. Visit 1 will entail fasting blood work, general health questionnaires, and introduction of the mCCapp. After reviewing the data from week 1,

participants who record <2 meals/day for ≥3 days or those with <6.5h self-reported sleep for ≥3days will be excluded from further study.

Randomization. The study statistician will generate the study randomization table prior to start of the study using SPSS program using block sizes of 4 and 6. He will be contacted by the study coordinator when a subject is ready to be randomized. Participants will be randomized into the interventional TRE arm or the control group with 1:1 ratios. All investigators involved in data analysis and collection will be blinded to the randomization of participants. Only study coordinators, statistician, and dietician will know which groups the participants are assigned to.

Visits (See Figure 1)

At visit 1 (day 1 - Screening), participants will be consented, have a fasting blood draw and be asked to complete general health questionnaires. The baseline laboratory tests for metabolic function (conducted at visit 1) include the following: CMP = comprehensive metabolic panel, CBC = complete blood count, TSH = thyroid-stimulating hormone, HbAa1c= glycosylated hemoglobin, hs-CRP= high sensitivity C-Reactive Protein, TG = triglycerides, LDL=Low density lipoprotein, HDL= high-density lipoprotein cholesterol, cholesterol assessed via NMR lipoprofile which will determine LDL Particle number. They will be introduced to the mCC app and instructed on its use. After visit 1, participants will enter a 1-week baseline period where they are instructed to use the mCC app to document all oral intake, exercise, and sleep quantity/quality.

At visit 2 (day 8 (with ± 3 days)-Baseline), they will return to the ACTRI for placement of CGM and actigraphy devices and to review the mCC app. They will also be asked to complete general sleep questionnaires. Those who meet logging criteria (recording >3 ingestion events of meals or beverages daily) and metabolic syndrome (based on blood work results, blood pressure, and waist circumference measurements) will be randomized into the standard of care group or TRE group. A muscle biopsy procedure and DXA scan will be performed at this visit as well for all participants regardless of group randomization.

At visit 3 (day 15 (with ± 3 days) - Intervention), participants will return to ACTRI to remove the CGM and return the actigraphy device. Each participant will meet with the dietician to discuss his/her eating pattern for the subsequent 12 weeks. For the TRE intervention group, the coordinator and the participant will select a 10 h eating interval that best suits their lifestyle based on his/her baseline eating pattern from the first week. The 10 h interval will be entered in the app, so that they can visualize their daily eating pattern and consume all meals within this interval. Participants will also be able to modify the 10 h window based on their schedule (e.g. if they eat a late dinner out at 8 PM then they will be given instructions on how they can resume eating at 8 AM the following day). TRE is the only intervention, and patients will not be instructed to change the quality, quantity, or caloric content of their diet in any way. 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. Both groups will continue to use the mCC app to document all dietary intake, exercise, and sleep quantity/quality for at least 1 week every month.

Between days 15-98, participants will enter the 12-week intervention period in which they will either be engaged in the TRE group or in the standard of care group. During this intervention period, participants of both groups will meet with the dietician over the phone twice (once during week 6 and once during week 10) to reinforce good nutritional practices. In addition, participants will receive push notifications on their phones (once daily) which will ask them to indicate whether they have eaten anything in the past 15 minutes (from time of response to the push notification). The purpose of these push notifications is to assess the false negative rate (food eaten but not documented), which accounted for 10.34% in the pilot study involving use of this smartphone app [50].

At visit 4 (day 92 with ± 5 days), participants will return for the application of CGM and the actigraphy device for one week. At visit 5 (day 98 with ± 5 days), participants will visit the ACTRI to return the CGM and actigraphy device. At this final visit, they will also have a fasting blood draw and be asked to complete the same general health questionnaires from visit 1. A muscle biopsy procedure and DXA scan will be performed at this visit as well. This will conclude the 12-week TRE intervention for the 14-week study period (Fig 1).

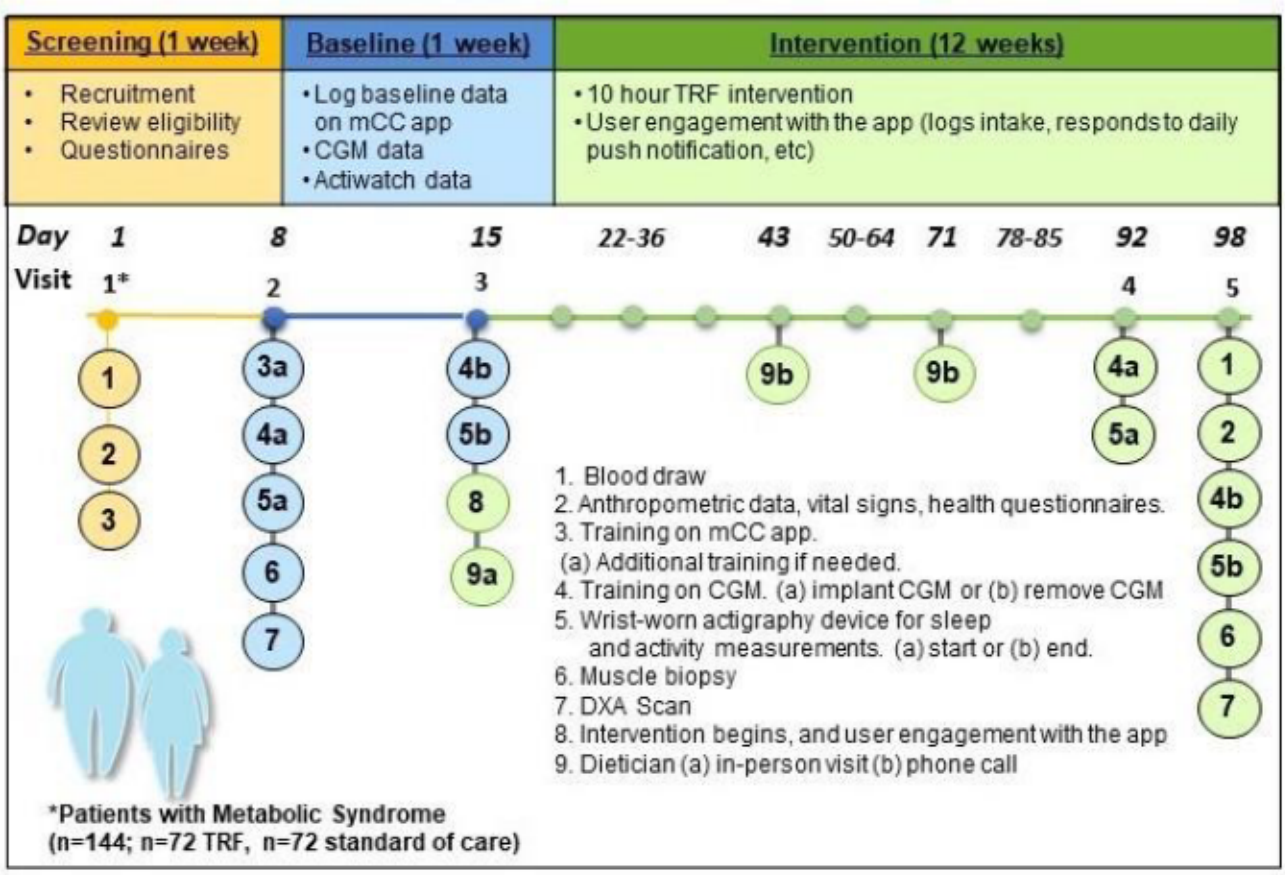

Figure 1. Timeline for participants. MCC app= myCircadianClock application. CGM = Continuous glucose monitor. DXA= Dual energy x-ray absorptiometry. Visit 1*= Participants will be disqualified if they do not meet inclusion criteria based on bloodwork, questionnaires, daily eating patterns, and adherence to the mCC app.

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 may be asked for additional measurements, blood work, muscle biopsy, and/or DXA scan at these optional follow-up visits.

Upon completing the study (or upon withdrawal) to avoid study bias, subjects will receive the results from the watch, CGM, DXA, and muscle biopsy data as an email attachment or printout. They will not be uploaded to the EMR. The lab results will be uploaded to the EMR as they are obtained from the lab. Subjects will already have access to the app data.

Procedures.

myCircadianClock application ("mCC app"). As it is increasingly becoming clear that the daily organization of activity, rest and eating pattern affect the circadian system and is also an output of circadian rhythm, longitudinal monitoring these parameters over days would produce important insight into circadian organization in humans. Co-I Panda has developed a smartphone app that combines both sensor-paired and

self-reported logging of food, sleep and activity. The mCC app (see figure 2) 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 two different arms (TRE and Control). After randomization, the coordinator will assign a participant to one of two arms and generate a unique activation code. Subjects in the TRE arm can set their daily eating periods and receive alerts and reminders specific to TRE protocols. The TRE groups will receive an automated alert 15 or 30min prior to the end of the eating interval to finish their last meal of the day. In the control cohort, the daily eating period customization will be disabled and they will receive standard of care prompts. All groups 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 from each cohort. For food entries, the user can annotate the food picture with food name and any other descriptor (portion size, left over etc.). Each cohort will be asked to record all food intake for at least one week every month for the duration of the study (14 weeks). Unlike other food tracking app, where each food logging takes an average of 40 seconds (myFitnessPal or Noom), the mCC app takes less than 10 seconds for logging one meal by using the picture feature. The simplicity of logging food metadata has led to good adherence with the app. Due to the fact that the app is only available in English, subjects are required to be fluent in English to properly use and read the information in the app.

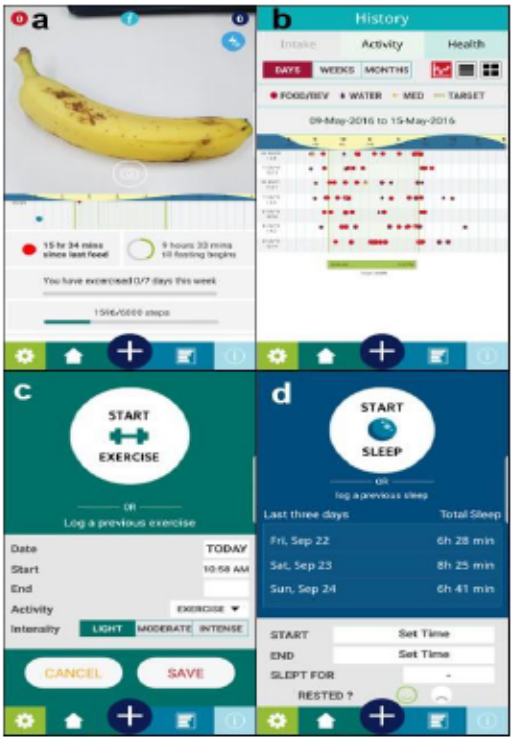

Figure 2. Overview of the mCC app

Questionnaires. 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).

Continuous glucose monitoring (CGM). All participants will be fitted with an Abbott Freestyle LibrePro® CGM, and be instructed on its use. CGM measures interstitial fluid glucose every 15 min for 14 full days, using subcutaneous sensor placed in the upper arm area. They will wear the CGM for one week prior to the start of the 12-week intervention period, and for one week prior to the end of the 12-week intervention. CGMs estimate blood glucose levels with high accuracy that correlates with those obtained from either venous or capillary blood [51]. Changes in the post-prandial glucose response (PPGR), mean amplitude of glycaemic excursion (MAGE), continuous overall net glycaemic action (CONGA), the glycaemic variability index (GVI) and standard deviation (SD) will be calculated. Average daily glucose level will be the primary endpoint.

Wrist-worn actigraphy. To measure habitual physical activity and sleep, participants will wear a Phillips accelerometer on their non-dominant arm for one week during week 1 and week 11. Wrist accelerometers have acceptable correlation (r=0.90) between daily physical activity and activity counts reported by accelerometers [52] and have been used reliably in NHANES [53]. The actigraphy data will be used to measure sleep onset, sleep duration and associated parameters (sleep efficiency and sleep fragmentation). Participants will also

complete a short sleep log every morning on the mCC app to record self-reported quality of sleep.

Cardiometabolic biomarkers: All participants will have their fasting blood drawn at visit 1 and visit 5 at the ACTRI by certified nurses. The baseline laboratory tests for metabolic function (conducted at visit 1) include the following: CMP = comprehensive metabolic panel, CBC = complete blood count, TSH = thyroid- stimulating hormone, HbAa1c= glycosylated hemoglobin, hs-CRP= high sensitivity C-Reactive Protein, TG = triglycerides, LDL=Low density lipoprotein, HDL= high-density lipoprotein cholesterol, cholesterol assessed via NMR lipoprofile which will determine LDL Particle number. An aliquot will be stored in peptidase and phosphatase inhibitor coated tubes for subsequent analyses of peptide hormones by multiplexed ELISAs. A total of approximately 1 cup (200ml or 16 tablespoons) will be collected from the subject for the entire study. There is the possibility of collecting more if the blood tests do not result due to lab and/or processing errors.

A blood repository will be built. Blood samples (plasma, serum) collected during the study will be stored and investigated at UCSD or the Salk Institute for the purposes of the study and also for possible future unforeseen biomarker testing such as neuroendocrine, inflammatory, and/or cardiometabolic testing. There is no intention to do genetic testing. Dr. Pam Taub will have solitary control of the stored specimens, which will be stored for an indefinite period of time. The samples will be labeled with a unique subject code as to de-identify and remove any PHI from the sample. The samples will be analyzed after subject completes study, and the analysis will be conducted in batches based on availability of resources. Samples will not be shared with researchers outside of those associated with this protocol.

Meals, activity and sleep. The smartphone application myCircadianClock (mCC app) will serve as an electronic food, activity, and sleep diary. On the server side, a sub-study dashboard will be created for this specific project. Clinical coordinators from Dr. Taub's lab and a research associate from Dr. Panda's lab will have password-protected access to the study data. In the study summary dashboard, the participant's study code and the date of activation of the app will be shown along with their daily log. If a participant fails to log any food data for more than 1 day, the dashboard flags the participant and sends an alert to the coordinator. The coordinators will login to the dashboard weekly to monitor food intake data, and follow up with flagged participants as necessary. The TRE participants will self-select a 10 h interval which they will consume all of their food and beverages. Their chosen eating interval is highlighted in the app so that they can easily track the progress of their daily eating within their set interval. For the standard of care groups, the participants' eating interval will be set to an eating pattern of >14 h. If any participant faces difficulty logging data, or has questions about any of the features of the app, they can contact the study coordinator through the feedback feature of the app. Their questions will be encrypted and delivered to a HIPAA compliant email server specifically set up for this study.

Muscle biopsy: Under local anesthesia, we will use a 5mm side-cutting needle for the percutaneous muscle biopsy. We will obtain 200-300mg of skeletal muscle from the lateral portion of the quadriceps femoris (vastus lateralis). A portion of the tissue will be immediately frozen in liquid nitrogen for subsequent RNA and protein extraction. A portion will be fixed in tissue fixative for microscopy analyses following a light microscopy technique optimized for muscle mitochondria [54] at the Salk Institute Waitt Biophotonics center using a ZEISS LSM 800 with Airyscan. Four different thin muscle sections from each sample will be imaged and analyzed. A stereological analysis to ascertain the ratio of mitochondrial volume to cytoplasmic volume will be performed. Point counting will be used to determine the mitochondrial volume densities by overlaying a grid on each digitized image. Mitochondria and cytoplasm lying under intercepts will be counted. The relative volume of mitochondria will be expressed as the ratio of intercepts coinciding with this organelle to the intercepts coinciding with cytoplasm. Mitochondrial membrane surface areas are to be measured using Image J software. Sample preparation, imaging and data processing will be performed by trained microscopy technicians at the Waitt Biophotonics Center.

Muscle samples collected during the study will be stored and investigated at UCSD or the Salk Institute for the purposes of the study and also for possible future unforeseen mitochondrial testing. There is no intention to do genetic testing. Dr. Pam Taub will have solitary control of the stored specimens, which will be stored for an indefinite period of time. The samples will be labeled with a unique subject code as to de-identify and remove any PHI from the sample. The samples will be analyzed after subject completes study, and the analysis will be conducted in batches based on availability of resources. Samples will not be shared with researchers outside of those associated with this protocol.

Dual Energy X-ray absorptiometry (DXA) scan:
A total of two DXA scans will be performed at the ACTRI, once at the beginning of the study before the intervention period and once at the end of the study after the intervention period. DXA body composition will be measured with a Hologic Discovery W densitometer, quantifying fat mass of the total body, arms, legs and trunk, abdomen, and hip. Participants lie supine on a table for six minutes during the scan. Changes in body composition such as greater muscle mass will be documented.

RNA analyses. The muscle will also be analyzed for changes in gene expression levels in Dr. Panda's lab at Salk. As observed in animals, TRE is hypothesized to change the expression or function of key transcriptional regulators of mitochondria biogenesis and function (PGC-1α FOXO1, SIRTs), lipid metabolism (REV-ERBs), protein folding (ATF6), and glucose metabolism (CREB). Furthermore, hypothesized changes in mitochondria number and function would reflect in changes in expression of key nuclear encoded mitochondria genes. Therefore, qPCR offers an inexpensive method to simultaneously quantify muscle gene expression. Additionally, some of the mitochondrial functional analysis will be done in the lab of Dr. Hemal Patel at UCSD.

Results will be validated by western blot analyses of protein levels. Total RNA will be extracted using RNeasy columns (Qiagen), following the manufacturer's instructions. Total RNAs from all samples will be processed in a single batch to reduce experimental noise. Reverse transcription followed by quantitative PCR (RT-qPCR) protocols using gene specific validated primer pairs will be used in triplicate reactions. Data normalization and quantification will be done using procedure followed in the Panda lab [55].

Protein analysis. Muscle protein will be extracted following standard protocol and protein levels will be quantified by multiplexed ELISA, or western blot using antibodies targeting specific markers of muscle or mitochondria function. Mitochondria function will be analyzed by citrate synthase activity using the Srere method [56].

Statistical Analysis.
Specific Aim 1 – Evaluate the impact of TRE on glucose homeostasis.
Hypothesis 1: The TRE group will have significantly lower blood glucose level compare to the control group at the end of the study.
Independent Variables: Group (TRE, Control Group), Week (with two levels of Baseline Week and Intervention Week), Day (with 7 levels of days 8-15 for Baseline Week and 92-98 for Intervention Week).
Dependent Variable: CGM.
Statistical Analysis: CGM data will be analyzed to determine changes in glucose response within individuals. Initially preliminary analyses will be performed for all hypotheses by examination of the distribution of variables to assess their characteristics (means, standard deviations, and skewness). Continuous measures will be tested for normality and homogeneity of variance. Non-normally distributed variables will be transformed to meet the normal distribution assumption for linear models. Randomization will be assessed by performing a series of Wilcoxon-rank sum tests, Chi-square or Fisher's exact tests to compare the groups on demographic and initial baseline clinical variables. Any variables on which the groups differ initially will be explored as

covariates in subsequent analyses. A daily average for glucose value will be computed for each week of study (total of 14 daily measurements). This hypothesis will be tested by using mixed effect model as described by Gibbons et al (1988) [57], Hedeker et al (1991) [58] and Laird et al (1982) [59]. The model will include a random intercept and random effect for Week and Day factors. A fully saturated treatment by Week by Day model will be utilized for inference. Co-variance structure will be chosen based on Akaikes Information Criterion (AIC). Denominator degree of freedom will be calculated using the Kenward-Roger small sample correction. The primary outcomes will be tested using an intent-to-treat framework and no subjects will be excluded from the analyses. Data will be analyzed using SPSS version 24. All analyses will be two-tailed, where applicable, with $\alpha = 0.05$.

Specific Aim 2 – Assess changes in metabolic biomarkers in response to TRE.

Hypothesis 2: TRE induced decreases in blood glucose levels will be accompanied by improvements in biomarkers such as LDL particle number via NMR lipoprofile, hs-CRP, and HbA1c, which reflect cardiometabolic stress at the end of the study.

Independent Variables: Group (TRE, Control Group), Day (Baseline: day 1 and Intervention: day 98).

Dependent Variables: *LDL particle number (via NMR lipoprofile), hs-CRP, HbA1c*.

Statistical Analysis: Data will be examined initially similar to hypothesis 1. This hypothesis will be tested using Repeated-Measured Analysis of Variance. All analyses will be two-tailed using Bonferroni correction method for multiple testing.

Specific Aim 3- Assess the impact of in body composition

Hypothesis 3: The TRE group will have significantly lower abdominal fat mass compare to the control group at the end of the study.

Independent Variables: Group (TRE, Control Group), Day (Baseline: day 8 and Intervention: day 98). Dependent Variables: *DXA*.

Statistical Analysis: Data will be examined initially similar to hypothesis 1. This hypothesis will be tested using Repeated-Measured Analysis of Variance.

Exploratory Aim 1 – Elucidate the effects of TRE on changes in mitochondrial structure and gene expression in skeletal muscle.

Impaired glucose homeostasis can result from insulin resistance, compromised mitochondrial function, adiposity, dyslipidemia, and inflammation. To elucidate the underlying mechanisms, skeletal muscle biopsies will be utilized to analyze 1) alterations in mitochondrial structure/function and 2) changes in gene expression of candidate target genes triggered by TRE. These exploratory analyses will be performed using Mixed effect model or RM-ANOVA.

Missing Data: Missing data values will be minimized by intensive training of the interviewers in techniques of clarifying answers and checking questionnaires while participants are on-site. When missing values are identified, we will employ several approaches. If at all possible, participants will be rescheduled or a tester sent to the individual's place of residence within 24 hours of completion of tests or interviews. Missing data will be examined to assess randomness. The pattern of missing data will be examined according to the procedure recommended by Little and Rubin [60] which includes comparing group differences in the primary outcomes of subjects with versus without missing data. The selected method of data analysis allows inclusion of subjects with missing data or those who terminated the study early, without relying on data imputation procedures. All laboratory-based measures will be reviewed for completeness immediately following collection and prior to the participant's dismissal. Despite our intensive protocol to limit participant attrition, we do expect missing data over the course of this study and have adjusted our recruiting sample accordingly. The primary outcomes will be tested using an intent-to-treat framework. If the extent of missing data is small and the data appear to be consistent with a missing-at-random model (MAR), then the maximum-likelihood analysis using all randomized cases and the observed data is an appropriate method for handling the missing data. In the MAR model the missingness can be a function of the observed covariates and observed outcomes. The MAR assumption is effective in treatment situations. The critical element when conducting MAR-based analyses is to

include variables related to the missingness in the statistical model. The distinction between ignorable (MAR) and non-ignorable missingness (missing not at random; MNAR) is generally not empirically testable and we acknowledge the possibility that data may be missing not at random. Therefore, we propose to perform MNAR sensitivity analyses using pattern mixture models [61]. Some patterns will likely have small numbers of cases precluding parameter estimation for these patterns separately. In this case we will apply the Hedeker and Gibbons approach [62] that combines missing data patterns to estimates model parameters that are conditional on the missingness using a binary variable in the model to denote missing data at one or more time points.

We will also test whether the drop-outs are random or systematic by comparing the drop-outs with the study completers on the baseline data. An absence of significant differences would support the random nature of drop-outs. Effect of drop-outs will be minimized by the following steps: First, we had selected data analysis method which allows the inclusion of subjects who drop-out or were terminated early in the study, without relying on data imputation procedures. Therefore, no subjects will be excluded from the analyses due to their study status. Second, effect of any systematic differences between the drop-outs and completers will be explored by including the variable of concern to the model as a covariate.

Power Analysis: The required sample size was calculated using G*Power software and for the mixed model approach using the RMASS program provided by Hedeker (http://tigger.uic.edu/~hedeker/ml.html). We are confident that the proposed sample size of 144 provides us with a minimum power of 80% to detect a medium effect size for all hypotheses. Medium effect sizes were selected based on previous studies that are reviewed above (see pages 4-5). For the mixed model, the medium effect size is defined as a between-group difference increasing linearly from 0 at baseline to .5 SD units at the last time point. The minimum power estimation is based on sample size calculation for 10% and 20% attrition, correlations of 0.2, 0.5, and 0.8 between the repeated measures, and for medium and large effect sizes.

Data Management: Data management and statistical analysis will be performed by the study statistician, Dr. Shahrokh Golshan. A comprehensive menu driven data management system will be developed for this project using Microsoft Access software. This system will provide a stable and secure platform for: a) study data input, validation, edit and query; b) subject tracking system for all subjects' study activities (screening and study visits); c) data storage, compilation, selection, and summary transfer to various statistical/graphical software and for institutional reports (IRB, DSMB); and d) data backup and archival control. All subjects are assigned a unique ID number. The patient names are never used in conjunction with the unique clinic ID number. The single name-to-ID relational file is kept in an encrypted form electronically and in a locked filing cabinet physically. Statistical analysis will be conducted using SPSS software package version 24. All statistical transfer routines are inherently secure via their operating platform and they contain no patient names or personal data.

10. HUMAN SUBJECTS

In order to enroll 144 adult volunteers (72 in the TRE arm and 72 in the control arm), we expect to screen 288 participants as in typical nutrition or behavioral intervention studies we screen 2 subjects to enroll 1. We anticipate a 20% drop-out rate with 120 participants completing the trial. We will recruit patients from ResearchMatch and the cardiology and internal medicine clinics at UCSD Medical Center; in addition, we will utilize the bioinformatics report generated from EPIC to assist in recruitment. Participants will be screened and must meet inclusion and exclusion criteria before they are enrolled in the study. The inclusion and exclusion criteria are below.

Inclusion criteria:

1. Age: 18-75 years
2. BMI $\geq$ 28 AND

3. metabolic syndrome, as defined as presence of 3 or more of the following criteria:
    - Elevated fasting plasma glucose ≥ 100 mg/dL
    - Elevated waist circumference:
        - In Asians: ≥ 90 cm in men, ≥ 80 cm in women
        - In all other races: ≥ 102 cm in men, ≥ 88 cm in women
    - Fasting plasma triglycerides ≥ 150 mg/dL, or on drug treatment for elevated triglycerides
    - Reduced High-density lipoprotein (HDL)-cholesterol <40 mg/dL in males or <50 mg/dL in females, or drug treatment for reduced HDL-cholesterol
    - Elevated blood pressure, Systolic blood pressure ≥ 135 mm Hg and/or diastolic blood pressure ≥ 85 mm Hg or drug treatment for hypertension
4. Own a smartphone (Apple iOS or Android OS)
5. Baseline eating period ≥ 14 hours/day
6. 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-hypertensive, anti-diabetes drugs), no dose adjustments will be allowed during the study period.

Exclusion criteria:

1. Taking anti-diabetic medications or insulin within the last 6 months
2. Manifest diabetes, defined as fasting glucose >126 mg/dL, HbA_{1c} > 6.5%, or diagnosis of diabetes
3. Known inflammatory and/or rheumatologic disease
4. Active tobacco abuse or illicit drug use or history of treatment for alcohol abuse
5. Pregnant or breast-feeding women
6. Shift workers with variable (e.g. nocturnal) hours
7. Caregivers for dependent requiring frequent nocturnal care/sleep interruptions
8. Planned international travel during study period
9. History of major adverse cardiovascular event within the past 1 year (acute coronary syndrome (ACS), percutaneous coronary intervention, coronary artery bypass graft surgery, hospitalization for congestive heart failure, stroke/transient ischemic attack (TIA)).
10. Uncontrolled arrhythmia (i.e. rate-controlled atrial fibrillation/atrial flutter are not exclusion criteria).
11. History of thyroid disease 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).
12. History of adrenal disease.
13. History of malignancy undergoing active treatment, except non-melanoma skin cancer.
14. Known history of type I diabetes,
15. History of eating disorder.
16. History of cirrhosis
17. History of stage 4 or 5 chronic kidney disease or requiring dialysis
18. History of HIV/AIDS
19. Currently enrolled in a weight-loss or weight-management program
20. On a special or prescribed diet for other reasons (e.g. Celiac disease)
21. Currently taking any medication that is meant for, or has known effect on, appetite
22. Any history of surgical intervention for weight management
23. Uncontrolled psychiatric disorder (including history of hospitalization for psychiatric illness)
24. Sleep apnea determined by the Epworth Sleepiness Scale (ESS)
25. Depression determined by the Beck Depression Inventory (BDI)
26. Failure to use the smartphone app for documentation (defined as <2 meals/day for ≥3 days during baseline) or with <6.5h self-reported sleep for ≥3 days during 1 week baseline period.

The main site of the interviews and clinical testing (vitals, blood draw, muscle biopsy, DXA scan) will be at the UCSD Altman Clinical and Translational Research Institute (ACTRI). Blood will be processed at the UCSD Clinical Laboratory before being transferred to Salk institute for storage and analysis.

11. RECRUITMENT AND PROCEDURES PREPARATORY TO RESEARCH

We will screen and recruit normal subjects and patients from UCSD. We will also utilize ResearchMatch. We will also be screening patients through EPIC, with the help of their bioinformatics department, for research subjects who might qualify for our criteria. EPIC Bioinformatics will help search for the following to generate a list of potential research subjects:

1. Patient's name, date of birth (DOB), and Medical Record Number (MRN)
2. Patient's primary care provider (PCP) name and contact information
3. Ages 18-75 for inclusion.
4. BMI $\geq$ 28 for inclusion.
5. Metabolic Syndrome
6. Waist circumference:
    a. In Asians: $\geq$ 90 cm (Men) or $\geq$ 80 cm (Women) for inclusion
    b. In all other races: $\geq$ 102 cm (Men) or $\geq$ 88 cm (Women) for inclusion.
7. Triglycerides $\geq$ 150 mg/dL (or on drug treatment for elevated triglycerides) for inclusion.
8. Reduced HDL-C < 40 mg/dL (Men), < 50 mg/dL (Women) (or on drug treatment for reduced HDL-C) for inclusion.
9. Elevated blood pressure, systolic blood pressure $\geq$ 130 mmHg and/or diastolic blood pressure $\geq$ 85 mmHg (or treatment with antihypertensive drug with history of hypertension) for inclusion.
10. Elevated fasting glucose $\geq$ 100 mg/dL (or drug treatment of elevated blood glucose) for inclusion.
11. No prior adverse cardiovascular events (ex: heart attack) for inclusion.
12. No prior cerebrovascular events (ex: stroke) for inclusion.
13. History of liver or kidney disease for study exclusion.
14. History or chronic infectious disease; autoimmune/inflammatory disease, cancer, COPD, anemia, type I diabetes, familial hypercholesterolemia, history of weight loss surgery, history of venous thromboembolism, cancer, malignancy, or psychiatric illness for study exclusion.
15. Depression or sleep apnea for exclusion.
16. History of smoking and/or illicit drug use and/or treatment for alcohol abuse for study exclusion.
17. Currently pregnant, for exclusion.

After we identify patients of interest for the study, we will contact their primary care provider or cardiologist and provide them with a letter with the following information: brief study description, research team contact information, and request to approach/contact subject. We will also ask the clinicians to specifically ask the interested patients if it is acceptable if one of our research team contacts them. If the patients are interested and give their consent, then our study coordinator will either contact them by email, phone, or clinic visit.

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 personal health information (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 research office at the Altman Clinical Translational Research Institute (ACTRI). 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 metabolic syndrome.

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 (see section 21), 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 metabolic syndrome.

Reconsenting

In the event that the consent is amended, subjects who are active and have not yet completed the study will be informed of these changes and be asked to sign the updated consent form. The updated consent form will be provided to them at an upcoming visit, or through mail, email or fax. They will be given time to review these changes and provide their written consent. They may sign and return it to the research team at the upcoming visit, or through mail, email or fax.

Subjects who have completed the study and are no longer active in the study will be made aware of the changes through mail, email or fax. However, these subjects will not be asked to acknowledge or confirm the receipt of the updated consent or to sign and return it.

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. 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. Dual energy x-ray absorptiometry (DXA) 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 DXA 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 your 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.
8. Muscle Biopsy: The risks of the muscle biopsy include pain, bleeding, bruising and infection at the biopsy site. These risks will be minimized with pressure and bed rest after the procedure. The risks of using local anesthesia may include temporary numbness, tingling sensations, and potential allergic reaction. You may feel some pain in your muscle when the tissue is removed. After the anesthetic wears off, the biopsy site may be mildly tender for 1-3 days. It is likely that the incision site will scar. The scar from the muscle biopsy will be about 1/4 inch long. There is a very small chance that the scars from the biopsy sites may become thick (hypertrophy).
9. Reduced daily eating period (from $\geq$ 14 hours per day to 10 hours per day): hunger, fatigue, and hypoglycemia
10. Fasting (for at least 12 hours prior to study visit) for muscle 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, and/or reapplication of the continuous glucose monitor if it fell off or did not record data.
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 as the patient identifier on study documents and samples. This document will be kept in a secure location in a locked cabinet. Only the PI and authorized research team members will have access to the cabinet.

15. RISK MANAGEMENT PROCEDURES AND ADEQUACY OF RESOURCES

Strict sterile technique will be observed during venipuncture and muscle biopsy. 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/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. Blood drawing will be minimized to no greater than 200 ml per patient. Subjects will be thoroughly informed of 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. Taub.

Monitoring procedures

Dr. Taub 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:

- Death
- Life-threatening
- Event requiring inpatient hospitalization or prolongation of existing hospitalization
- 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. Taub 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
2. Wrist-worn actigraphy device:
    a. Skin irritation.
3. 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.
4. DXA Scan
    a. Low dose radiation
5. Muscle Biopsy
    a. Pain
    b. Bleeding
    c. Bruising
    d. Infection
    e. Anxiety/fear
6. Reduced daily feeding period (from $\geq$ 14 hours per day to 10 hours per day)
    a. Hunger
    b. Fatigue
    c. Hypoglycemia
7. 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).

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. Taub, who is a cardiologist, will be overseeing the procedure to check for the patient's vital signs. Dr. Taub'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 (ACTRI) is equipped with trained medical staff (physicians and nurses) who can respond quickly. Should an event occur, Dr. Taub 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. Taub 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. Muscle Biopsy
    a. Prior to enrollment into the study, the patient will be educated on the risks of getting a biopsy from their leg muscle. Dr. Taub 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 muscle biopsy will be thoroughly sanitized with an alcohol wipe prior to the incision with scalpel and the needle stick. Before the procedure is performed an area of skin and the deeper tissues on the outer front portion of either one of the patient's lower thighs will be numbed using anesthetic (xylocaine) and then the small incision will be made with the scalpel. The special biopsy needle will be inserted through the incision and into the thigh muscle with extreme care several times and a small amount of tissue will be removed (about the size of a small pea). Deep pressure will be applied on the area for 20 minutes to minimize the risk of bleeding and then a steri-strip tape will be used to close the incision. Dr. Taub, who is a cardiologist, will be performing the procedure, she has performed over 200 muscle biopsies with no complication. Dr. Taub'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 (ACTRI) is equipped with trained medical staff (physicians and nurses) who can respond quickly. Should an event occur,

Dr. Taub 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. Taub will monitor the patient until the problem has been resolved or has stabilized.

4. 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 protocol established by the device manufacturer: 1) Wash hands with soap and water, 2) Choose sensor insertion site on upper arm, 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, 6) Once sensory filament is inserted into the subcutaneous layer, an adhesive product may or may not be used. 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 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 (ACTRI) is equipped with trained medical staff (physicians and nurses) who can respond quickly. Should an event occur, Dr. Taub 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. Taub will monitor the patient until the problem has been resolved or has stabilized.
5. DXA Scan
    a. Prior to enrollment into the study, the patient will be educated on the risks of obtaining a DXA 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 DXA 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. Taub 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. Taub will monitor the patient until the problem has been resolved or has stabilized.
6. Reduced daily feeding period (from $\geq$ 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 or type II diabetes on medical therapy. 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. Taub will monitor the patient until the problem has been resolved or has stabilized.
7. 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. Taub 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 electronic forms and will be identified with the study's subject ID. 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

Patient privacy and confidentiality will be addressed by adhering to data securing measures. These measures include use of a password protected, secure, internet-based database management program.
All study forms including consents, HIPAAs, and case report forms (CRFs), which will include elements from the present and past history, patient demographics, including name, medical record number, age, current medications and lab draws/results, will be stored in in a locked cabinet in Dr. Taub's research office in the ACTRI building. When not in use CRFs will be stored in a locked cabinet or office within a limited-access facility. Access to the CRFs will be given to study staff only. Information collected by study staff for study purposes will be kept confidential and will not be shared with anyone, including study participants and sponsors, outside of the study

staff or IRB. To minimize the potential loss of confidentiality and privacy, patients will be assigned a unique number as their subject identifier code. The unique subject code will be used to label all study documents. This information will only be available to study personnel and will be secured in the same manner mentioned above.

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. Taub's research office in the ACTRI building. Samples will also be sent to Dr. Panda's lab at the Salk Institute for storage and analysis.

The research team will uphold the standards of HIPAA and patient privacy as allowed by law. The following Personal Health Information will be collected for research purposes: name, date of birth, medical record number, phone number, email, address. The following will also be collected through the mCC app: travel plans, timestamp of food/beverage intake and geographic location data.

mCC app data: Daily nutrient dates will be collected from each subject. The user's sleep and activity data may be collected by sleep log questions in the app and by passive monitoring of accelerometer in the phone. When the subject starts eating they will launch the app on the phone and take a photograph of the item(s) consumed. Beverages and water are also considered as food for the purposes of this study. The app includes an optional provision to append a brief textual message to accompany the photograph, in case the subject intends to add any remarks regarding the photograph that may not be obvious from the photograph itself (i.e. Brand names, size, etc.).

For any reason, if the subject does not wish to record a photograph of the food item(s), he or she has the choice of:

- choosing from a list of foods commonly consumed and the approximate time that food was consumed by the subject
- typing out the details of what he or she ate and the time that food was consumed

These two options can be also be utilized to retroactively log the food consumed in case the subject forgets to take a photograph at any mealtime.

Upon pressing send, the photograph (which is sent as a JPEG file) and/or the textual content (which is sent as a text file) is transmitted to a cloud server. The URL for this server is Circadianstudy.org and the name of the file that is transmitted to us is of the format:
<unique id>_<date>_<time>.<file extension (jpg or txt)>
e.g. 2b6f0cc904d137be2e17_2011-08-15_11-08-36.jpg

The files received in the server will be regularly monitored by the investigators to ensure the smooth progress of the study. In particular, if a user forgets to log the data for two to three standard meal times, he or she will be sent a push notification reminder automatically by the server.

User input: The overall layout of the data input, storage and analyses is shown in Fig 3. The default and recommended mode of collecting data will be taking a picture of any food or beverage an individual ingests. As soon as the app is activated, it will seek the instantaneous geolocation (longitude, latitude). The individual can take a picture of the food, can choose to use the picture or retake and makes sure there is no personally identifiable item in the picture (ID card, another person's face at a conference table, etc). Once s/he is satisfied with the quality of the picture, the individual will have the option of adding a short annotation for the item. Such optional text entry will describe the food, (item name, decaf or sugar free, portion size, whether the

picture represents leftover from the previous meal etc.). Every textual entry will populate an individualized database of foods or beverages consumed. Thus, the app will learn the subject's entries, so that over time, it can auto-suggest these annotations. The first time the app is launched it creates and stores a 20 character alphanumeric identifier for the device, e.g. Bw2iAm67juEglgN1qK8t. This identifier is unique and permanent for the duration of the study, stored on the device as well as the server and serves the purpose of uniquely but anonymously keeping track of multiple users.

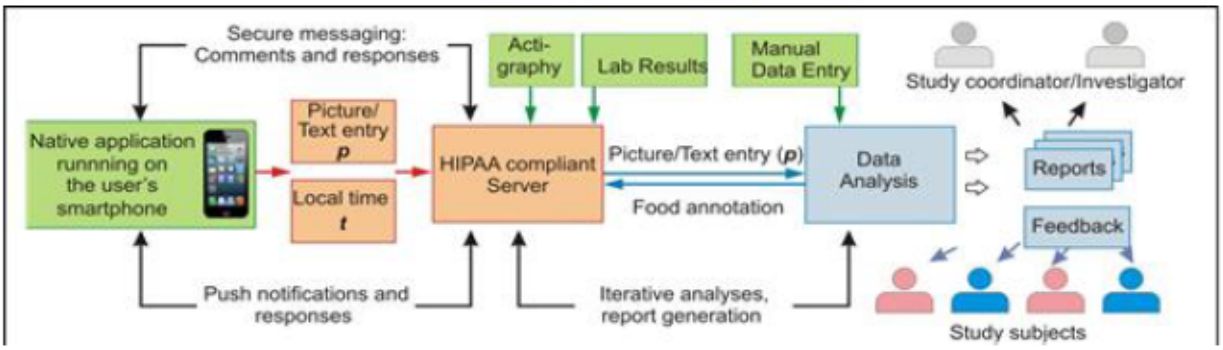

Fig 3. Overall layout of the data acquisition, storage, and analyses plan.

The second option is to scan a barcode or a QR code of a food/beverage/medication item. Scanning the code simplifies downstream data annotation. Food manufacturers are required to label or offer detailed nutritional information. The app's barcode scanner is used to retrieve the manufacturer's data during the subsequent annotation step.

The third option is to manually type (text entry) the food item. This feature will be mostly used for situations where taking a picture of the food is not socially acceptable or if the subject forgets to record an event. As soon as the individual fills out the text field, a time wheel will automatically pop up asking whether the event is current or happened in the past. The current version of the app does not have a barcode scanner and the text and camera functions are in different tabs. In the proposed version all three modes will be integrated into one screen and the end-user can seamlessly switch between these data gathering modes.

After the picture/barcode/text is entered, the user will choose whether the item is "water", "beverage", "Food" or "Medication/supplements". This simple act will sort the items into four major classes of ingested items and greatly simplify downstream annotation. At the beginning of the study, the participants will be encouraged to annotate, at least once, any medication or supplements they are taking in the text field of a picture. This data-sorting at user-end is a new feature over the current prototype.

Data transmission: As the user annotates the picture, the app will name the picture or text file with a unique identifier. The identifier will begin with the user ID followed by the timestamp. Network congestion can sometimes slow down the data transmission, cause frustration and reduce compliance. In the current app this varies from less than a second to a minute, during which the screen freezes. Therefore, the picture/text along with the time stamp and geo-location will be stored in a temporary onboard cache while the network functionality is getting restored, and once the app is able to connect to the internet, it can transmit the data to the server. All picture files are reduced in size by a factor of 10 or 15 to make the final image file size <300kb. At this size the pictures have enough resolution for identifying food items, while minimizing the data transmission time to one-tenth. Each outgoing data package from the smartphone will have three pieces of information; **(1)** a picture or textual entry <subject identifier>_<sorting tag>_<date>_<time> e.g. Bw2iAm67juEglgN1qK8t_f_2011-11-11_18-12-58.jpg **(2)** annotation text e.g. Bw2iAm67juEglgN1qK8t_2011-11-11_18-12-58.dsc and **(3)** Location tag e.g.

Bw2iAm67juEglgN1qK8t_2011-11-11_18-12-58.loc

Personalized push notification: Preliminary data has indicated that personalized push notifications (which appear as a badge resembling that for text messages) few times a week improves compliance with data collection. Push notifications are widely employed by most apps and most users are well-versed with their usage and utility. The geo-location tag will be used to calculate the local sunrise-sunset time and the reported events in the past 2 days will be used to compute the time period when the subject is likely active. A simple push notification will be automatically sent to the individual asking "Did you eat or drink anything in the last 30 min?" with a two option "Yes/No" response. To account for the possibility that there might be a time lag between when the push notification is sent and when the user notices it on the phone, the time interval used for generating the yes/no question will encompass the 30 min period prior to the user noticing it (and not when the push notification is sent by the server). The subject's response (Yes or No) along with the presented question and the time at which it was answered will be transmitted back to the server where it will be compared with the timestamp of data packages received within the last 30 min (this time interval will need to be optimized in trials). Such push notification and response gathering serve two purposes. First, push notification improves participants' engagement with the app and improves reporting over extended period of time. Second, it helps obtain an error estimate for the individual participants. Concordance between an event in the given period and response is expected, but discordance will be taken into consideration for error estimates. There are two major types of errors: when a person recorded an event, but answered no and when a person did not record an event and answered yes. In the second case, the subject will be prompted to text the missed event.

Sleep log: Although sleep and physical activity will be passively monitored with the smartphone's accelerometer, a tab in the app will encourage users to input their self-reported sleep parameters as has been extensively used in many peer reviewed studies. Throughout the study participants will take the Pittsburgh Sleep Quality Index (PSQI) questionnaire. PSQI's administered in one month interval differentiates transient sleep problems from persistent problem and has a test-retest reliability of >80%. Combination of PSQI, periodic post-sleep questionnaire, and accelerometer will be powerful tool to evaluate sleep and activity. During the course of the study the subject will be encouraged to go to the sleep log tab before going to bed where s/he will simply push the button – "going to bed" and every morning the app will automatically switch to the sleep window, so that the participant can push "woke up" and rate his/her sleep on a 4 step sliding scale from "Very good" to "Very bad" (same as Q6 of PSQI). If the subject reports bad sleep, Question 5 of PSQI asking probable cause of bad sleep will appear. During the day, the subject will also be asked to record any nap by clicking two buttons "going to nap", "finished napping". These answers are sent to the server along with geo-tag and timestamp. Such engagement with the subject serves two purposes – to get a better estimate of person's self-reported sleep and a better estimate of the geo-location of the subject's "home". We anticipate in the next several months the smartphones will improve their accelerometer function, so that activity and sleep can be passively measured.

Database architecture: The files and databases will be hosted on a commercial cloud computing platform: Amazon Web Services (AWS). AWS provides EC2 (Elastic Compute Cloud), a platform for HIPAA compliant on-demand computational capacity via virtual machines called "instances" that range from low capacity to very high capacity in terms of CPUs, RAM and storage. Further, the computational capacity can be rapidly and conveniently scaled up or down as needed. We will instantiate 2 large capacity Linux servers (main server and backup) with load balancing to capture the data that the smartphone users transmit. The data will be stored on AWS S3 (Simple Storage Service). These two servers will remain online 24h a day, 7 days a week. A third EC2 server will regularly retrieve incremental data from the main server, run processing and analytics jobs, create and dispatch mTurk tasks and provide visualization of the data to the study coordinators. We will use the relational database MySQL to store data and PHP (programming language) to interact with this database. The following tables will be maintained in one database: (1) table to store the file names for the data package triplet:

picture/text, description as entered by the user, geo-location (2) alphanumeric identifiers generated for each user at the time of activation of the app, the user's study start date and his/her Apple push notification ID used to send push notifications (3) the times at which push notifications were triggered by the server and (4) the user responses to those, and (5) the responses to the sleep and behavioral questionnaires. A second database will record the annotations for each image. To maintain data integrity and prevent data loss, all data received from study participants as well as that residing in the MySQL databases will be mirrored on the backup AWS instance.

Image analyses: State-of-the-art image recognition algorithms cannot reliably identify food items, so manual annotation of the food is necessary. Presorting of the food types to water, medication/supplements and food/beverages by the app users will streamline image analyses. From the preliminary study we find up to 30% of events involve water intake. The rest 70% of images were largely calorie-containing food or non-water beverage items. Lastly, a small fraction is nutrient supplements and medications. Crowd sourcing may be used to annotate the collected images to identify the food and beverage items and extract nutrient information. A "gold standard" reference dataset comprise of images obtained from previous participants and annotated by a trained dietician/nutritionist will be administered to every crowdsourcing annotator. This gold standard dataset will be a reference platform for estimating the reliability of the individual's annotations. Crowdsourcing as a first step has several advantages. The Amazon Mechanical Turk (mTurk) platform enables recruiting as many or as few individuals spread all over the globe for an extremely low cost. Crowdsourcing can be done 24/7 and is cost-effective. Since a large crowd can be mobilized to a given task, thousands of food pictures can be annotated within days or even hours. More than half of the food and beverages consumed by free-living individuals are prepackaged food or prepared meals from a fast-food or normal restaurant. Collecting nutrient information from these food items involves look-up tables from CDC or Nutrient Data Laboratory (http://www.ars.usda.gov/main/site_main.htm?modecode=12-35-45-00). Food is cultural, has rich diversity and food complexity is constantly changing. So, information on complex or food unique to a culture or geographic location is more likely to be annotated faster by first obtaining a provisional annotation by the local "crowd" and subsequent verification by nutritionists. Each food or beverage item will be first named and various parameters will be estimated, including calories, volume, macronutrient content, pre-packaged/prepared or home-cooked, fruits and vegetables, taste modality (sweet, sour, bitter, salty, umami), alcohol content and type (wine, beer, spirit). Water annotation serves two purposes; to estimate the daily fluid intake by individuals and to estimate supplemental intake through bottled water (Vitawater™, Smartwater™ etc). Medications and nutrient supplements are difficult to annotate based on picture alone. So, at the end of the monitoring period, a sampling of medication pictures which cannot be annotated will be sent back to the participants for identification. Although the intervention is based on time of eating, the picture annotation will help evaluate whether changing the eating pattern indirectly improves nutrition quality and reduces total calories by enabling individuals, for example, to reduce after-dinner snacks, desserts or alcohol.

Geo location: Geo-location of food intake offers insights to the eating pattern, living environment and lifestyle of individuals. Collection and analyses of this information can improve both personalized nutritional and lifestyle recommendation as well as help in public policy formulation. As the geographic information system maintained in local and federal government becomes digitized, annotations for each geolocation can eventually be automated in the future. However, for the current project, we will rely on crowdsourcing for annotating each geolocation. Each geolocation will be mapped using a web-based mapping program. The median resolution of geo-location (latitude and longitude coordinates) in our pilot study has been 30 ft. Geolocations collected at evenings and nights and those associated with response to sleep questions will be clustered to find the subject's "home" as the location (within ~300 ft. resolution) with the maximum number of night time events. Similarly, the cluster of weekday daytime geolocations (within ~300 ft.) resolution will be annotated to find approximate "work" location. The remaining geolocations represent other locations including transit, restaurants, other residential place (friend/family) places of socialization, gym, parks etc. Some subjects may not have a well-

defined "work" address. Finally, the geo-tag classifier will be linked to the food annotation. As a result we can classify whether a food was consumed at home, work or other location and accordingly quantify various parameters of food with location.

Daily Activity Rhythms/Sleep Quality

Although several smartphones have inbuilt accelerometer and growing number of people use wrist worn commercial accelerometers, heterogeneity in the type of accelerometers and communication method between the app and the meter may not allow us to collect activity data from all participants. When this communication is feasible through APIs and SDKs, we will collect activity/sleep data from the device.

Optional data fields:

The app will have optional data fields to record mood, hunger level, alertness/sleepiness level, blood pressure, heart rate, bowel movements, pulse oximetry, temperature, ketone bodies (urine and or blood sample), and results from blood samples including: blood glucose, total cholesterol, low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), triglycerides, Hemoglobin A1c, Fibrinogen, C-Reactive Protein, and Homocysteine. A few controlled on a small size study population have reported insufficient sleep and erratic daily eating pattern can increase risk for metabolic diseases or worsen prognosis of these diseases. By collecting these additional parameters, we will be able to test in a large population if erratic eating pattern and insufficient sleep correlate with poor health status.

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 metabolic syndrome. The potential benefit to the individual to those randomized to the TRE group is that by engaging in TRE, subject may see overall improvement in cardiometabolic risk factors and decreases and biomarkers, which may improve subject's overall health and reduce risk for cardiovascular disease. There may also be improvements in sleep quality and mood. There may be no benefit to those randomized into the standard of care group.

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 patients with metabolic syndrome, including weight loss.

19. EXPENSE TO PARTICIPANT

There will be no expense to participants.

20. COMPENSATION FOR PARTICIPATION

Patients will be compensated with up to $400 for fully participating in the study. Parking for the study visits will be paid for by the research team. We do not provide compensation for cell phones, cell phone data, or Wi-Fi used for the app.

Participants will receive the following compensation per visit:
Visit 1-$40
Visit 2- $150
Visit 3-$30
Visit 4-$30
Visit 5-$150

21. PRIVILEGES/CERTIFICATIONS/LICENSES AND RESEARCH TEAM RESPONSIBILITIES

Pam Taub, MD (Principal Investigator) is an Associate Professor of Medicine at UCSD and is board certified in internal medicine and cardiology. She has clinical privileges at UCSD Medical Center. She 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.

Satchidananda Panda, PhD (Co-Investigator) is a researcher at the Salk Institute for Biological Studies. He will be responsible for the following aspects of the project design, data analysis, manuscript writing, and mCC app oversight.

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

Hemal Patel, MD, PhD (Co-Investigator) is a Professor of Medicine at UCSD. He will be responsible for the following data analysis and manuscript writing.

Michael Wilkinson, MD (Co-Investigator) is a cardiovascular disease fellow at UCSD. He will assist with the following: project design, patient recruitment, data analysis and manuscript writing.

Jia Shen, MD (Co-Investigator) is an Assistant Professor of Medicine at UCSD and is a board-certified cardiologist. She will assist with the following: patient recruitment, data analysis and manuscript writing.

Iwona Swiatkiewicz, MD, PhD (Co-Investigator) is an Associate Professor of Medicine at Nicolaus Copernicus University and a board-certified cardiologist. She is a visiting scholar who will assist with the following: patient recruitment, data analysis, and manuscript writing.

Adena Zadourian, BS (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, and manuscript writing.

Hannah Lo, BS (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: patient recruitment, patient scheduling, IRB submissions, administrative and technical support, data collection and analysis.

Meena Kaurani, BS (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: patient recruitment, patient scheduling, IRB submissions, administrative and technical support, and data collection and analysis.

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

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UCSD is the prime recipient (Salk Institute with Dr. Satchidananda Panda is subcontracted under us)
UCSD Fiscal Contact: Paula Maguina-Mercedes

24. BIOLOGICAL MATERIALS TRANSFER AGREEMENT

MTA is under review

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.

3. Table of IRB Amendments and ClinicalTrials.gov Updates

Amendment	IRB Approval Date	Date updated on Clinicaltrials.gov
UCSD IRB tentative Approval - pending IRB approval at Salk	10/12/2018	N/A
Full UCSD IRB approval	2/13/2019	N/A
1. Adding a short title ("The TIMET Study") to the long title. 2. Changed the number of clinic visits from 5 to 4 (combined visits 2 and 3). 3. Changed baseline from 1 week to 2 weeks. 4. Added Insulin as a measure to assess glucose regulation. 5. Updated the statistical days to reflect the current study design. 6. Updated the potential risk and management of using an ambulatory blood pressure monitor. 7. Updated compensation to reflect 4 visits. 8. Updated study personnel 9. Updated fund manager	2/22/2019	Included in the original Clinicaltrials.gov submission
Added new Flyers for Recruitment	3/6/2019	N/A
Added new Flyers for Recruitment	4/4/2019	N/A
Expanded analysis of muscle samples	4/16/2019	Included in the original Clinicaltrials.gov submission
First Participants Started Baseline – 4/29/2019		
Updated study personnel	5/6/2019	N/A
First Participant Stated Intervention – 5/14/2019		
1. Expanded inclusion criteria to allow BMI ≥25 (currently ≥28). 2. Included optional MRI scan of the liver for a subset of participants. 3. Updated study personnel and contact info. Note: The amendment was submitted before the enrollment of the first participant.	6/3/2019	Included in the original Clinicaltrials.gov submission
Updated study personnel (contact information change on CT.gov June 2020)	8/15/2019	6/12/2020
1. Added SOC participants to have an optional follow-up (same as TRE). 2. Added option of magnetic resonance elastography (MRE) along with MRI as approved because of added efficiency in measuring the amount of fat in the liver compared to an MRI alone. 3. Added another potential visit for participants whose labs are not recent enough to determine their eligibility for the study.	10/16/2019	N/A
Exclusion criteria have been revised to increase the cutoff value of the HbA1c >6.5% to HbA1c >6.8%, unless diagnosed with diabetes, to allow for the 0.3% margin of error in lab readings.	12/9/2019	1/26/2021
Changed questionnaires from paper to HIPAA-compliant Google forms	6/16/2020	N/A
Updated study personnel	6/18/2020	N/A
Added pamphlet for recruitment	7/10/2020	N/A
Expanded inclusion for glucose to have a fasting glucose of >	8/6/2020	1/26/2021

100 mg/dL or HbA1c >5.8 <6.9%		
1. Added recruitment through social media (ex. Facebook) and the option to record video of participant experience for recruitment. 2. Updated exclusion to exclude participants who traveled with a time zone change of 3 hours or more (currently excluded international travel). 3. Updated inclusion to allow for depression score on BDI if well-controlled.	9/15/2020	1/26/2021
Expanded blood glucose inclusion criteria requirement to be Elevated fasting plasma glucose ≥ 100 mg/dL OR HbA1c ≥ 5.7% < 7.1% The glycated hemoglobin criteria is being changed to allow for the inclusion of participants with an HbA1c as low as 5.7% rather than the current 5.9 %.	10/23/2020	1/26/2021
1. Inclusion Criteria: changed from a baseline eating period of ≥ 14 hours/day to ≥ 12 hours/day. 2. Updated study personnel	12/3/2020	1/26/2021
Allowed participants from the same household to be randomized to the same group.	12/23/2020	N/A
Sample Size Change. Goal to complete 118 with the option to randomize up to 144.	5/25/2021	6/17/2021
1. Sample Size: Fixed wording to reflect planned randomization (not completion) to 118, with the option to randomize up to 144, and updated statistical plan. 2. Primary End Point changed to delta in HbA1c between groups. The primary outcome was previously fasting blood glucose. Changed because HbA1C is a more widely used measure of glycemic control and fasting blood glucose can be variable from one day to another. 3. Inclusion/Exclusion: increased BMI allowed from 40 to 41 4. Added possible adverse event: vasovagal event during muscle biopsy. 5. Removed ambulatory blood pressure monitor as it was not included in the final study design. 6. Updated study personnel	12/2/2021	6/17/2021 and 7/20/2022 (enrollment number) and 12/10/2021 (primary endpoint and inclusion/exclusion) Others were not applicable
Updated study personnel	1/16/2022	N/A
Updated study personnel	7/19/2022	N/A

4. Justification for Revised Statistical Plan

The statistical plan was revised due to new analysis methods that were developed after the original statistical analysis plan was written for the CGM computation. The original plan of a mixed-effects model was selected to assess daily glucose levels gathered from the CGM. Instead, we used novel software (PMID: 34282646) that allowed for additional insight into the CGM data as a full data set, including measures of glycemic variability, that could not be attained for individual daily assessments alone. Using this method, daily measurements were combined and the CGM data were reduced to only two-time points, thus changing our analysis to a mixed Repeated Measures ANOVA.

Given that we had two time points for all outcomes, the analysis of all completed data was a more appropriate analysis than imputing data based on a single time point. This analysis was also supported by our assessment of baseline measures from participants who completed or withdrew. Missing data from participants who withdrew from the study were examined for randomness using 3independent t-tests to compare (1) all withdrawals from all completers, (2) within-group withdrawals vs completers, and (3) within-group withdrawals vs randomly sampled equal number of completers (Supplemental Tables 1-3). We found no significant differences between groups except in the MODD assessment from CGM. This difference was only seen when comparing all withdrawals from all completers, but not from within-group assessments or random sampling of within-group assessments. Thus, it is more accurate to assess all available data.

5. Revised Statistical Plan

Statistical Analysis.

Specific Aim 1 – Evaluate the impact of TRE on glucose homeostasis.

Hypothesis 1: The TRE group will significantly decrease HbA1c compared to the control group at the end of the study.

Independent Variables: Group (TRE, Control Group), Time (with two levels of Baseline and End of Intervention)

Dependent Variables: HbA1c.

Statistical Analysis: HbA1c data will be analyzed to determine changes in glucose response within individuals. Initially, preliminary analyses will be performed for all hypotheses by examination of the distribution of variables to assess their characteristics (means, standard deviations, and skewness). Continuous measures will be tested for normality and homogeneity of variance. Non-normally distributed variables will be transformed to meet the normal distribution assumption for linear models. Randomization will be assessed by performing independent t-tests to compare the groups on demographic and initial baseline clinical variables. Any variables on which the groups differ initially will be explored as covariates in subsequent analyses. The hypothesis will be tested using Repeated-Measures Analysis of Variance (aka Mixed ANOVA). The primary outcomes will include all available data. Data will be analyzed using SPSS version 28 (or the current version). All analyses will be two-tailed, where applicable, with $\alpha = 0.05$.

Sub-aim 1A: Assess changes in other glycemic measures including fasted blood glucose, insulin, HOMA-IR, and continuous glucose monitors (CGM).

Hypothesis: The TRE group will have a significant improvement in glycemic parameters (assessed via fasting glucose, HOMA-IR, Insulin, and CGMs) compared to the control group and compared to baseline.

Independent Variables: Group (TRE, Control Group), Time (with two levels of Baseline and End of Intervention)

Dependent Variables: Fasting glucose, Fasting Insulin, HOMA-IR, and glycemic control assessed via CGM (including measures of glycemic variability such as CONGA and MODD)

Statistical Analysis: Data will be examined initially similar to hypothesis 1. This hypothesis will be tested using Repeated-Measured Analysis of Variance.

Specific Aim 2 – Assess changes in cardiometabolic biomarkers in response to TRE.

Hypothesis 2: TRE-induced decreases in blood glucose levels will be accompanied by improvements in cardiometabolic biomarkers at the end of the study.

Independent Variables: Group (TRE, Control Group), Day (Baseline: day 1 and Intervention: day 98).

Dependent Variables: Cardiometabolic Biomarkers

Statistical Analysis: Data will be examined initially similar to hypothesis 1. This hypothesis will be tested using Repeated-Measured Analysis of Variance.

Specific Aim 3- Assess the impact of TRE on body composition

Hypothesis 3: The TRE group will have significantly lower abdominal fat mass compared to the control group at the end of the study.

Independent Variables: Group (TRE, Control Group), Day (Baseline: day 15 and Intervention: day 98). Dependent Variables: *DXA*.

Statistical Analysis: Data will be examined initially similar to hypothesis 1. This hypothesis will be tested using Repeated-Measured Analysis of Variance.

Missing Data: Missing data values will be minimized by intensive training of the interviewers in techniques of clarifying answers and checking questionnaires while participants are on-site. When missing values are identified, we will employ several approaches. If at all possible, participants

will be rescheduled or a tester sent to the individual's place of residence within 24 hours of completion of tests or interviews. Missing data will be examined to assess randomness. The pattern of missing data will be examined according to the procedure recommended by Little and Rubin [62] which includes comparing group differences in the primary outcomes of subjects with versus without missing data. The selected method of data analysis allows the inclusion of subjects with missing data or those who terminated the study early, without relying on data imputation procedures. All laboratory-based measures will be reviewed for completeness immediately following collection and prior to the participant's dismissal. Despite our intensive protocol to limit participant attrition, we do expect missing data over the course of this study and have adjusted our recruiting sample accordingly. The primary outcomes will be tested using an intent-to-treat framework, including all available data. If the extent of missing data is small and the data appear to be consistent with a missing-at-random model (MAR), then the maximum-likelihood analysis using all randomized cases and the observed data is an appropriate method for handling the missing data. In the MAR model, the missingness can be a function of the observed covariates and observed outcomes. The MAR assumption is effective in treatment situations. The critical element when conducting MAR-based analyses is to include variables related to the missingness in the statistical model. The distinction between ignorable (MAR) and non-ignorable missingness (missing not at random; MNAR) is generally not empirically testable and we acknowledge the possibility that data may be missing not at random. Therefore, we propose to perform MNAR sensitivity analyses using pattern mixture models [63].

We will also test whether the drop-outs are random or systematic by comparing the drop-outs with the study completers on the baseline data. An absence of significant differences would support the random nature of drop-outs. The effect of drop-outs will be minimized by the following steps: First, we selected a data analysis method that allows the inclusion of subjects who drop out or were terminated early in the study, without relying on data imputation procedures. Therefore, no subjects will be excluded from the analyses due to their study status. Second, the effect of any systematic differences between the drop-outs and completers will be explored by including the variable of concern to the model as a covariate.

Power Analysis: The required sample size was calculated using G*Power software We are confident that the proposed sample size of 118 provides us with a minimum power of 80% to detect a medium effect size of 0.55 for all hypotheses. Medium effect sizes were selected based on previous studies and fasting glucose data from 12 participants who had elevated glucose from our pilot study.