Title: Pilot Study of Time Restricted Feeding as a Weight Loss Intervention
NCT03571048
Last updated: 10/14/2020

Protocol #: 18-0487
Project Title: Pilot study of time restricted feeding as a weight loss intervention in overweight and obese adults
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Version Date: 10/14/2020

I. Hypotheses and Specific Aims:

The circadian timing of energy intake (EI) has emerged as a key factor in the regulation of body weight[1,2]. Studies have suggested that eating meals later in the evening or during the biological night when the circadian system is promoting sleep adversely influences the success of weight loss therapy[2-7]. In contrast, restricting EI to a short window during waking hours and extending the length of the overnight fast (i.e., time restricted feeding, TRF) may be a practical and useful strategy for promoting weight loss and weight maintenance[1,8,9]. However, potential benefits of adding TRF to a weight loss program have yet to be evaluated in a well-controlled clinical study. The overall objective of this proposal is to provide a foundation to inform the design of a future large-scale trial to evaluate the efficacy of TRF in generating weight loss. Our overall hypothesis is that feasibility, adherence, and acceptability of a weight loss intervention using TRF in the setting of a reduced calorie diet (RCD) – RCD+TRF - will be similar to compliance with an intervention using a reduced calorie diet alone (RCD), suggesting acceptability for a future large-scale trial. In this 12-week pilot and feasibility study, 60 overweight and obese individuals will be randomized 1:1 to RCD+TRF (EI restricted to a 10-hour window starting 1 hour from habitual waking time) or standard RCD (no restriction on feeding duration). The total duration of the intervention is 9 months. Measures include feasibility, acceptability and adherence to the interventions, body weight, body composition (DXA), EI (smart phone application), physical activity (PA, accelerometery), glucose variability (continuous glucose monitoring, CGM), sleep (questionnaires and polysomnography), and nocturnal substrate metabolism (room calorimetry). The specific aims (SA) are:

Specific Aim 1a. To evaluate the feasibility and acceptability of a 9-month TRF intervention compared to a standard dietary weight loss intervention (i.e. RCD). Feasibility of enrollment and retention, and acceptability of the intervention will be assessed in adults with obesity meeting inclusion/exclusion criteria proposed for the future large-scale trial. We will assess adherence to the weight loss programs, as measured objectively with a novel smartphone application and verified with CGM data, and subjectively with the use of questionnaires. We expect adherence to the RCD+TRF intervention will be similar to the RCD group, suggesting acceptability for a future large-scale trial. If we find differences in adherence between programs, the data will be used to optimize the RCD+TRF program prior to the future large-scale trial.

Specific Aim 1b. To assess the efficacy of RCD+TRF compared to RCD alone in producing weight loss at 12 weeks and reducing the risk of weight regain after 6 months of follow-up. Although the number of subjects included in this pilot study does not provide sufficient statistical power to detect differences in weight loss between groups, the data obtained will provide estimates of variability and effect size to assist with power and sample size calculations for the future large-scale trial.

Exploratory Aim. To explore potential mechanisms contributing to any observed differences between groups we will evaluate (A) objective measures of free-living behavior (energy intake, physical activity, and sedentary behavior) and (B) objective measures of glucose variability, sleep and energy metabolism Preliminary data on free-living behaviors and

in-laboratory measures of sleep and energy metabolism will allow us to further optimize a future trial and probe mechanisms to explain any weight loss differences between groups.

II. Background and Significance:

The question of whether *when* we eat is as important as *how much* remains unanswered and is critical in the development of future recommendations regarding body weight regulation. Altering meal frequency has been evaluated as a weight loss strategy due to epidemiological studies showing an inverse relationship between meal frequency and adiposity[10,11], but interventional studies have shown conflicting results, with no definitive evidence of differences in weight loss or metabolic risk factors[12-15]. Breakfast eating is also recommended due to evidence linking breakfast skipping with obesity[16-20]. However, several interventional studies have failed to show weight loss with breakfast eating[21-23]. On the other hand, in two weight-loss interventions, individuals who consumed more calories in the morning as compared to the evening lost more weight, even though both EI and self-reported PA were similar[2,3]. In addition, food intake at night (as seen with shift work), is linked to obesity independent of EI[24].

There are physiologically plausible mechanisms by which restricting EI to a window aligned with the early part of the day (i.e. TRF) may enhance weight loss and result in more favorable metabolic outcomes. A number of metabolic systems are entrained to circadian rhythms, including hormone, lipid and glucose concentrations, intestinal lipid absorption, and autonomic nervous system activity[25]. Restriction of EI to a window of time and/or extension of the fasting period may alter diurnal patterns and circadian rhythms in glucose and lipid metabolism in a manner that favors decreased obesity risk. In rodent studies, metabolic benefits were seen with a TRF protocol, despite equivalent EI of a high fat diet[8,9]. Only two studies of TRF have been done in humans. Gill and Panda[26] studied 8 overweight men and women with habitual eating duration >14 hours who were asked to restrict EI to a self-selected 10-11 hour window. Subjects lost weight (-3.27 kg), despite the fact that they were not specifically told to decrease their EI. Similarly, LeCheminant et al[27] studied 29 healthy young men in a cross-over design with a period of night eating restriction (no EI from 1900 to 0600 hrs) vs control, without recommendation to decrease EI, and found a modest weight reduction after 2 weeks (-0.4 kg). However, these studies were small and not designed as weight loss interventions.

This pilot study proposal is highly significant because we plan to address the gap in knowledge regarding the effects of EI timing on weight loss, which was recently highlighted as a priority research area by the NIH[28]. Results may have substantial influence on evidence-based weight-management recommendations and guidelines. While caloric restriction is a necessary component of all weight loss regimens[29], most individuals struggle to adhere to a reduced calorie diet long-term[30,31]. Studies of different weight loss diets show significant variability in weight loss success[30], and patients may need to try multiple different strategies before finding one that is successful and sustainable. In the era of individualized medicine, the ability to choose the most suitable dietary strategy for an individual patient would increase the likelihood of successful weight loss.

Innovation: We believe there are 4 innovative aspects to the proposed studies. ***First*, TRF has yet to be evaluated as a weight loss strategy**. The ultimate goal of assessing dietary patterns is to identify those patterns which may be able to effectively produce weight loss, so a clinical study assessing weight loss outcomes is critical to determining the relevance of this line of research. While many studies have evaluated weight loss interventions with variations on number of meals and/or macronutrient content of the diet, studies evaluating the timing of food intake are notably lacking. ***Second*, TRF has not been evaluated within the context of a behavioral weight loss intervention that includes a moderately reduced calorie diet and behavioral support to facilitate adherence[32]**. Provision of a comprehensive program will likely enhance adherence to TRF and result in greater weight loss. Developing a TRF behavioral support curriculum is innovative and will inform the delivery of the intervention during the larger trial. ***Third*, the project seeks to develop a number of novel approaches to study the effects of TRF on free-living behaviors**. For example, we propose the use of a smartphone application with photographic food

records in combination with continuous glucose monitoring (CGM) to capture free-living EI and monitor adherence to the program. In addition, we will evaluate the effects of TRF on free-living PA and sleep. In the Bath Breakfast Project, individuals were found to have greater physical activity energy expenditure when consuming as compared to skipping breakfast[21,23]. Also, sleep restriction alters food intake and habitual physical activity[33], but less is known about how dietary patterns affect sleep. Food intake near the onset of sleep is negatively associated with sleep quality,[4-6] so it may be hypothesized that the early TRF paradigm could improve sleep. **Finally, we will measure sleep and nocturnal energy metabolism in a subset of subjects (n=5) in each group (RCD and RCD+TRF) following the intervention and correlate these measures with longitudinally determined weight regain at 6 months**. These studies will allow us to start collecting data on physiological processes that might promote weight regain or support weight maintenance following weight loss. TRF studies in rodents show favorable adaptive responses to nocturnal nutrient oxidation that appear to be protective against metabolic challenges such as high fat feeding[8,9,26]. If these adaptive responses in nocturnal nutrient metabolism observed in rodents translate to humans, then TRF may be a potent strategy for promoting weight maintenance in reduced obesity, the most vexing problem facing obesity treatment.

III. Preliminary Studies/Progress Report:

The idea to study TRF comes from our group's experience with breakfast skipping studies[22,34]. While our study[22], as well as other interventional trials[21,23] failed to show weight loss with breakfast skipping, these studies did not evaluate the timing of food intake throughout the rest of the day. In contrast, we[34] and others[35] have shown that breakfast skipping was acutely associated with an increased insulin response to lunch, and other studies have shown increased glucose variability[21] and insulin resistance[23] with breakfast skipping. This, along with emerging research relating to the meal timing and the circadian regulation of metabolic systems[36], provides a physiological basis for potential metabolic benefits of TRF.

IV. Research Methods

A. Outcome Measure(s):

Primary Outcomes:

- **Feasibility and tolerability of the interventions** assessed by enrollment and retention numbers, protocol related adverse events (AE's), and questionnaires assessing satisfaction, quality of life and mood.
- **Adherence to interventions** assessed both with self-report and with the use of a smartphone application in conjunction with continuous glucose monitoring.

Secondary Outcomes:

- **Weight (scale) and body composition (DXA)** obtained at baseline (prior to the weight loss intervention), at 12 weeks (following the weight loss intervention), and at a 6mo follow-up time point.
- **Physical activity** assessed by accelerometry
- **Free-living sleep** patterns assessed by wrist actigraphy
- **Objective (in-laboratory) sleep** recording using Compumedics Inc Siesta digital sleep recorders
- **Meal timing and energy intake** assessed by photographic food records
- **Patterns in 24h glucose** assessed by continuous glucose monitors over 7 days before and after the weight loss intervention
- Questionnaire based measures of **appetite**
- **HgbA1c and lipids**
- **Resting metabolic rate and nocturnal energy metabolism** by indirect calorimetery

B. Description of Population to be Enrolled:

Inclusion criteria.

- Adult males and females with a BMI of 27-45 kg/m^2 and weight stable over the previous 6 months;
- Age, 18-50 years old;
- Passing medical and physical screening, and analysis of blood and urine screening samples;
- Typical eating duration >12 hours per day (assessed by questionnaires);
- Own a smartphone
- Reside or work within approximately 30 minutes driving distance of CU-AMC (potential participants living outside of this driving distance could be included in the study at the PIs discretion).
- For participants in the optional Reproductive Hormone Sub-study: females, age 18-40, with regular menstrual cycles (between 18-40 days).

Exclusion criteria.

- Pregnancy or lactation for women (women who are >6 months postpartum with no plans of becoming pregnant in the next year and who are not currently lactating can be included; oral contraceptives will be allowed if medication has been consistent for the prior 6 months)
- Postmenopausal women (menopausal status will be assessed during the history and physical, with requirement of self-reported regular menstrual cycles for the last year; women who have undergone hysterectomy but with ovaries in place who continue to have regular menstrual symptoms can be included)
- Being considered unsafe to participate as determined by the study physician;
- History of cardiovascular disease, diabetes, uncontrolled hypertension, untreated thyroid, renal, hepatic diseases, dyslipidemia or any other medical condition affecting weight or lipid metabolism;
- History of human immunodeficiency virus or hepatitis B or C (self-report);
- Taking medications affecting weight or energy intake/energy expenditure in the last 6 months, including weight loss medications, antipsychotic drugs or other medications as determined by the study physician;
- Having abnormal blood chemistry (eGFR<45mL/min, AST or ALT >3 times the upper limit of normal) or as deemed significant by the study physician;
- Being a smoker or having been a smoker in the 3 months prior to their screening visit;
- Working night shifts;
- For participants completing the PSG studies, extreme early or extreme late chronotype as determined by the Munich Chronotype questionnaire[37]. Participants completing the primary weight loss intervention will not be excluded based on chronotype;
- Night eating syndrome (at least 25% of food intake is consumed after the evening meal and/or at least two episodes of nocturnal eating per week) as assessed with meal pattern assessment questionnaire;
- For participants completing the PSG studies, greater than moderate sleep apnea (score high risk ≥ 2 or more categories on the Berlin Questionnaire). Participants completing the primary weight loss intervention will not be excluded based on Berlin OSA risk scores.
- For participants completing the PSG studies, use of medications affecting sleep (benzodiazepines and other sleep aids, as determined by study physician). Participants

completing the primary weight loss intervention will not be excluded based on use of medications affecting sleep.

- Binge eating behaviors as identified by a score of >17 on the Binge Eating Scale (BES)[38].
- For participants in the optional Reproductive Hormone Sub-study: Current use of hormonal contraceptives, or use of hormonal contraceptives during the 4 months prior to the start of the weight loss intervention, previous diagnosis of polycystic ovarian syndrome.

C. Study Design and Research Methods

Informed consent, medical screening, labs, questionnaires, and baseline metabolic rate assessment.

Informed consent, medical evaluation and labs. After telephone screening, interested participants will be scheduled for an in-person screening visit to determine eligibility. After providing written informed consent, participants will undergo the following evaluations: medical history and physical examination (including measurement of weight, height, BP, HR), and screening tests (Hemoglobin A1C, lipid panel, comprehensive metabolic panel, TSH and pregnancy test). Patients who have had these tests done and found to be normal within the last 6 months may submit the results and forego the screening labs. If any screening test is abnormal the participant will be informed immediately and asked to follow-up with his/her doctor.

Questionnaires. Participants will be asked to complete a meal pattern assessment questionnaire to assess for components of night eating syndrome, as well as the Munich Chronotype Questionnaire[37] to evaluate chronotype. In addition, participants will complete the Binge Eating Scale (BES), a 16-item survey used to identify the presence and severity of binge eating behaviors[38].

Resting energy expenditure (REE): REE will be measured after an overnight fast using standard indirect calorimetry[39] (Parvo Medics TrueOne 2400, Salt Lake City, UT).

To enhance feasibility of recruitment, participants can complete the informed consent and history and physical exam during a separate visit from the screening/baseline procedures that require fasting (blood draw and REE measurement). In addition, participants may undergo DXA testing at the screening visit if screening is done within 4 weeks of the intervention start date.

Baseline Assessments (7-day period no less than 4 weeks prior to starting the weight loss intervention).

EI and meal timing will be measured using photographic food records by utilizing the camera function of participants' smartphones to take a picture of the food or beverage. Participants will be provided with detailed instructions regarding methods of taking food photographs, as outlined by the CTRC Nutrition Core. After taking the photographs, participants will text photographs directly to either a Meallogger account or a Google Voice account (note: an amendment was submitted in 1/2020 to switch to Google Voice after data collection using Meallogger in Cohorts 1 and 2 were already completed.). Subjects will be asked to record every intake event for 7 continuous days. The data from this 7-day recording period will be used to assess timing of energy intake in both groups, and adherence to time restriction in those patients in the RCD+TRF group. In addition, this information will be compared with data from continuous glucose monitoring to determine whether glucose excursions exist in the absence of recorded food intake events. Based on published data[40] showing that mean post-prandial glucose excursions in patients with normal glucose tolerance are ~40.5 mg/dL, this threshold will be used to indicate an episode of food intake. EI will be estimated using the food images collected during the last 3 days of the 7 day recording period by staff in the CTRC Nutrition Core, as previously described[41,42].

Glucose variability: Free-living plasma glucose levels will be measured with a glucose monitoring

system (FreeStyle LibrePro, Chicago, IL) for 7 continuous days coinciding with the cell phone-based meal tracking described above. The device consists of a sensor applied to the back of the patient's upper arm that measures glucose in interstitial fluid every 15 minutes.

Patterns of PA and Sedentary Behavior: The activPAL3 micro (PALTechnologies, Glasgow, Scotland) will be used to estimate time spent in moderate-to-vigorous PA (MVPA), light activity, and sedentary behavior over a 7-day period at baseline. The activPAL3 micro uses accelerometer-derived information about thigh position to estimate time spent in different body positions. The time-stamped "event" data file from the activPAL software will be used to determine time spent sitting, standing, and stepping per day.

Appetite will be measured using visual analog scales (VAS) before and after each meal for the final 3 days of cell phone meal tracking as previously described[43] and as used by our group in prior studies[34,44,45].

Free-living Sleep Patterns: Subjects will complete several validated questionnaires to evaluate aspects of daytime sleepiness, sleep quantity/quality, and sleep timing: Berlin Questionnaire, Epworth Sleepiness Scale[46], Pittsburgh Sleep Quality Index[47], and Morningness -Eveningness Questionnaire[48]. Each subject will also keep a written log of sleep/wake times and wear a wrist activity and light exposure recorder to document free-living sleep/wake patterns for 7 continuous days (Actiwatch Spectrum, Philips Respironics, Bend, OR).

Mood, quality of life and eating behaviors: Quality of life (QOL) will be assessed with the RAND-36[49,50] physical and mental health component summary score. Mood state will be assessed with the Profile of Mood States 40 Item Questionnaire (POMS-40)[51-53]. Eating behaviors will be assessed with the Three Factor Eating Questionnaire (TFEQ)[54].

Body Weight and Composition Assessment. Body weight and composition will be measured using a digital scale and dual energy x-ray absorptiometry (DXA, Hologic Inc., Bedford, MA).

Resting energy expenditure (REE): the resting metabolic rate measurement would be repeated in any participant presents with significant weight gain or loss (+/-2kg) between the time screening and the time of baseline assessments. Otherwise, the REE measurement performed at the time of screening will be considered as the baseline.

Optional In-laboratory assessments:

Nocturnal energy metabolism: Participants will be invited to spend a night in the UCH whole room calorimeter for the measurement of nocturnal energy metabolism. We aim to perform approximately n=5 room calorimeter studies per group depending on budget. To facilitate recruitment for the optional procedure, the evening spent in the room calorimeter could be completed any night following study enrollment and prior to starting the weight loss intervention (except for during the 7-day free-living assessment of meal timing, EI, and activity described above). Prior to the room calorimeter stay, participants will be asked to perform 3-days of sleep logs and cell phone based meal logging with time stamps to ensure meal and sleep timing are consistent prior to the chamber stay. Subjects will be provided an energy balance diet (calculated as RMR times an individually determined activity factor) on the day of the overnight chamber stay at baseline. The macronutrient composition of the diet will be the same at baseline and at the 12-week follow-up: carbohydrate = 50%, fat = 35%, and protein = 15% (percent daily caloric intake:25% breakfast, 30% lunch, 35% dinner, and 10% afternoon snack). The meals will be eaten over a 12 hour period as follows: subjects will be asked, "when can you eat your first meal in the morning? (1, 2, or 3 hours after waking?)". This clock time will be T0. The meal times are then set at +5 (lunch) , +8 (snack). +11.5 (dinner) after T0. . Subjects will arrive at the CTRC at least 2 hours before dinner and be placed in the room calorimeter at their habitual bedtime for measurement of nutrient oxidation and energy expenditure as previously described[55]. Substrate oxidation will be calculated from urine nitrogen excretion, O_2 consumption and RQ based on published equations[56]. The chamber stay will be repeated at the 12-week follow up (between

weeks 12 and 16). The energy content of the diet will be determined based on RMR measured at baseline and reduced by 10% (as per weight loss intervention dietary prescription). In addition, participants in the TRF group will be asked to eat all food within their chosen 10-hour eating window as follows: where their habitual breakfast time is T0, Lunch=+4h, snack=+6h, and dinner=+9.). Participants in the RCD group will eat all meals within the same 12-hour eating window as was followed at baseline. The *rationale* for measuring nocturnal metabolism is that previous studies (including recent studies from our group) show that nocturnal energy expenditure and fat oxidation are independent predictors of longitudinal weight change. It is likely that the RCD+TRF and the standard RCD interventions will have differential effects of nocturnal fuel partitioning which could predict rates of weight regain in the groups.

Polysomnographic (PSG) sleep recording and scoring. During the night spent in the room calorimeter subjects will be fitted with Compumedics Inc Siesta digital sleep recorders. The PSG study will consist of an electrocardiogram (ECG) and electroencephalogram (EEG), electromyogram (EMG), electrooculogram (EOG), and respiratory bands placed over the chest and abdomen. The sleep study will be scored according to revised AASM guidelines. Sleep latencies will be defined as follows: sleep onset latency (SOL), time from lights out to the onset of three continuous epochs of PSG-defined sleep; latency to persistent sleep (LPS), time from lights out to the onset of 10 continuous minutes of PSG-defined sleep; latency to slow wave sleep (SWSL), time from LPS to SWS; and REM sleep latency (REML), time from LPS to REM sleep. Sleep stages (stage 1 sleep, stage 2 sleep, SWS, and REM) will be matched with EE values offset by 2 min to account for the lag-time response of the whole-room calorimeter relative to the timing of the sleep recording.

Behavioral Weight Loss Program (Weeks 1-39).

Following completion of the baseline assessments, all participants will receive a 9-month group-based behavioral weight loss program, with randomized groups meeting separately. Participants will be randomized 1:1 to RCD+TRF or RCD. Groups will be held at the AHWC and will be taught by an RD with experience in leading group-based behavioral weight loss interventions. In case of situations in which in-person classes cannot be held (i.e., due to concern for transmission of disease during the COVID-19 global pandemic), meetings will be held virtually via Zoom. Zoom is a HIPAA compliant web and video conferencing platform. All participants will be provided with a link to join the meeting and instructions regarding optimal conditions in which to join the meeting (i.e., a quiet location with minimal distractions and ability to devote full attention to the meeting). Group meetings will be moved back to in-person when it is deemed safe to do so. Group meetings will take place weekly during weeks 1-12, then monthly between months 4 and 9. Groups will meet separately to maintain intervention integrity. Classes will last ~60 minutes and the curriculum will be delivered using a mix of large group discussion, small breakout discussions, visual demonstrations, and written exercises. The programs will fulfill all recommendations for behavioral interventions outlined in current guidelines[32]. Current guidelines suggest the most effective behavioral treatment for obesity is a high-intensity, comprehensive weight loss intervention provided in individual or group sessions by a trained interventionist with principle components of a moderately reduced calorie diet, increased PA, and behavioral support to facilitate adherence [32]. Curriculum will be based on the Colorado Weigh behavioral weight loss program, which uses a skills-based approach and cognitive behavioral strategies for lifestyle modification with a dietary focus on daily caloric restriction[57,58]. The Colorado Weigh curriculum includes topics addressing the importance of exercise for weight loss. Specifically, the recommendations are for participants to achieve 150 min/wk of exercise, but exercise will not be supervised. Curriculum for RCD+TRF will be developed by Dr. Thomas, with input from a registered dietitian and will feature similar weekly themes as RCD, but modified to focus on support specific to TRF.

Dietary and Activity Prescriptions. Both groups will focus on caloric restriction to decrease overall EI. Participants will be given a personalized calorie goal based on their measured RMR. If RMR by indirect calorimetry and by Mifflin St-Jeor equation differ by >10%, then the average of the 2 values will be used. This estimated RMR will be reduced by 10% and then rounded to the nearest 100 kcal

Suggested macronutrient content (55% carbohydrates, 15% protein, 30% fat) will be the same for both groups. Participants in the RCD group will not be given any specific instruction regarding timing of food intake. Participants in the RCD+TRF group will be instructed to eat only during a window of 10 hours, starting within 3 hours of waking. They will also be instructed in specific strategies to support TRF including: strategies to deal with hunger outside eating windows, distraction techniques, and choosing a balanced diet/appropriate portions during feeding windows. All participants will be counseled on the importance of PA for weight loss and will receive a recommendation to perform 150 min/wk of PA.

Self-Reported Dietary Adherence, Effort, and Self-Efficacy: At weeks 4, 8 and 12, participants will be asked to rate on a 1-10 Likert scale: 1) how adherent they were to prescribed study diet over the past week, 2) how hard was it to adhere to prescribed study diet over the past week, and 3) how likely they feel they can adhere to prescribed diet for the next month[30]. These questionnaires will also be administered monthly from month 4 through 9 to determine whether participants have continued to follow their prescribed dietary pattern. In addition, participants will be asked to use the Meal Logger app (Cohorts 1 and 2) or Google Voice at weeks 4 and 8, and monthly between months 4 and 9 in order to objectively assess adherence to the dietary intervention.

Intervention Safety and Tolerability: Participants will be asked to report AEs to study staff as they occur, to be reported to our IRB as per institutional guidelines. We will collect additional parameters to assess tolerability at week 12 and at the 6 month follow up visit. Quality of life (QOL) will be assessed with the RAND-36[49,50] physical and mental health component summary score. Mood state will be assessed with the Profile of Mood States 2 (POMS-2)[51-53]. Eating behaviors will be assessed with the Three Factor Eating Questionnaire (TFEQ)[54].

Intervention Acceptability: Satisfaction with both the standard RCD and RCD+TRF program intervention will be assessed at week 12 and month 9 with a questionnaire that asks participants to rate on a 1-10 Likert scale how satisfied they were with specific domains of the intervention. The major domains that will be assessed are satisfaction with: 1) ability to adhere to caloric restriction and time restriction (for RCD+TRF group), 2) content of the dietary support curriculum, 3) content of the exercise support curriculum, 4) group meeting frequency and structure, and 5) weight loss achieved to date. Participants will also be asked in open-ended fashion to provide feedback on each domain of the intervention. We will also assess intervention acceptability by comparing the frequency of protocol related AE's, requirement for protocol modification, and attrition between groups.
In addition, at the end of the intervention, participants in both the TRF and RCD groups (n=6-8 per group) will be asked to participate in focus groups led by Dr. Masters, a licensed clinical health psychologist with experience developing heath focused behavioral interventions and conducting focus group research. The participants will be required to fill out an additional consent form. If the study team is unable to meet with them in person to obtain consent, then a virtual consent will take place via ZOOM. The goal of the focus groups will be to obtain more specific information about their perceptions of, and experiences with, the intervention. Participants will specifically be asked to discuss, via open-ended inquiry and discussion: 1) aspects of the intervention itself that were difficult or relatively easy to follow; 2) barriers that limited their adherence or satisfaction; 3) beneficial aspects of the intervention; 4) factors that facilitated their ability to adhere to the intervention; 5) aspects that they would change and their recommended changes. To obtain a more complete understanding of the participants' experiences, extensive efforts will be made to include individuals who were non-adherent or dropped out of the interventions. A research assistant will attend the focus groups to act as a scribe, and the sessions will also be recorded using an audio recording device which will be later used to transcribe the entire session. Once the recording has been transcribed, the audio recording will be deleted. Data in response to the open-ended queries will be analyzed by the focus group leader using primarily inductive procedures, searching for themes that could inform intervention refinement in advance of a larger clinical trial. The focus groups will last 60-90 minutes and will be conducted at the Anschutz Health and Wellness Center.

In case of situations in which in-person classes cannot be held (i.e., due to concern for transmission of disease during the COVID-19 global pandemic), meetings will be held virtually via Zoom. Zoom is a HIPAA compliant web and video conferencing platform. All participants will be provided with a link to join the meeting and instructions regarding optimal conditions in which to join the meeting (i.e., a quiet location with minimal distractions and ability to devote full attention to the meeting).

Adherence: Adherence to the prescribed study diet will be objectively assessed through review of meal logs collected with the MealLogger app and Google Voice account as described above, as well as through evaluation of glucose excursions in comparison with the meal logs in order to determine if glucose excursions exist in absence of recorded food intake. In addition, adherence will be assessed subjectively as described above through the use of questionnaires using Likert scales to address ability to adhere to the study diet.

Post-intervention assessments. Within 3 weeks following the initial 12-week weight loss intervention all participants will complete 7 days of free-living EI recording (cell phone app), PA recording (accelerometers), sleep recording (wrist actigraph), appetite recording (VAS), and glucose monitoring (CGM) as described above for the baseline assessments. Participants will also have repeat assessments of HgbA1c, lipid panel, resting metabolic rate and body composition (DXA) during an outpatient CTRC visit. Participants will also repeat questionnaires as described above: adherence to the dietary intervention, QOL (RAND-36), mood state (POMS-2), eating behaviors (TFEQ), and sleep questionnaires (Berlin Questionnaire, Epworth Sleepiness Scale, Pittsburgh Sleep Quality Index, and Morningness -Eveningness Questionnaire). Participants completing the optional in-laboratory room calorimeter and objective sleep measurements at baseline will be asked to complete follow-up measurements. The follow-up measurements of sleep and nocturnal energy metabolism can be completed on any evening starting at week 10 of the intervention not to exceed 4 weeks following completion of the intervention. The only exception is that the inpatient visit cannot occur during the 7 days of free-living EI, PA, and glucose monitoring.

COVID-19 Post Intervention Changes

Due to the COVID-19 pandemic, we are unable to carry out week 12 measures as described above. Instead, the study team will drop off the accelerometer and wrist actigraph to participant's homes. The packet of devices will be left on the participant's doorstep to eliminate person to person contact. Proper PPE will be worn by the study team during the drop-off and pick-up time including gloves and masks. The participants will be asked to wait a few days before wearing the devices and encouraged to wipe each device down before placing it back in the packet for pick-up. Once the participants have worn the device for 7 consecutive days, the study team will pick up the devices from the doorstep and wipe down the packet with a disinfecting wipe. The devices will not be handled by the study team for at least 1 week post pick-up. The devices will be handled as follows:

- The study team will wear gloves and a mask any time they are handling devices
- Devices will be disinfected with a Cavi-wipe before being placed in the individual device packets
- Each device will be placed in its own sanitized plastic bag before being placed in the device packet
- Devices will be given to the entire cohort on one date, so that there will be no sharing of devices between participants
- No study team member will handle devices if they are exhibiting symptoms of COVID-19 or have been exposed in the last 14 days.

These measures will be put into place so long as the orders from campus, local, state, or federal government are such that the study measures cannot be carried out as originally planned. The study team will resume normal visit procedures once it is safe and permitted to do so.

We will still collect all non-contact outcomes such as the appetite recording (VAS), and the 7 days of food logging via Google voice. In addition to these measures, for the cohort participating in the weight loss intervention during the COVID-19 pandemic only, we will administer a questionnaire specific to COVID-19 (changes in work, levels of motivation, ability to adhere to the diet, etc) as well as the Perceived Stress Scale, a validated psychological instrument for measuring the perception of stress. CTRC measures such as the DXA, blood panel, and resting metabolic rate will not be collected under these circumstances.

6-month follow-up assessments. Participants will continue to attend monthly group meetings during months 4-9. At 6-months following the completion of the weight loss intervention all subjects will be asked to come into the CTRC for repeat measures of RMR, body weight (scale) and composition (DXA). In addition, they will be asked to repeat questionnaires assessing adherence to the dietary intervention, satisfaction with the intervention, QOL (RAND-36), mood state (POMS-2), eating behaviors (TFEQ), and sleep (Berlin Questionnaire, Epworth Sleepiness Scale, Pittsburgh Sleep Quality Index, and Morningness -Eveningness Questionnaire, and Munich Chronotype Questionnaire). Due to the switch we made to ZOOM classes mid-intervention, we will send out a survey to the participants that asks about their experience with the online platform and how it compared to their in-person classes. We have also amended the focus group questions to ask about this as well.

Optional Reproductive Hormone Sub-study Procedures: Urine Collection to Evaluate Reproductive Hormones. These measures serve as markers of hypothalamic-pituitary-ovarian (HPO) axis function, which is evidenced to be suppressed in both women with obesity, as well as women with low energy availability (i.e., restricting energy intake or exercising intensely), which is the cornerstone of behavioral obesity treatment. Funding for these measures is provided by an NIH K award (HL 143039; PI Caldwell). Data from these participants will be combined with that being collected in another weight loss trial at the University of Colorado, Anschutz Medical Campus (COMIRB, 17-0369, PI, Catenacci) for statistical analysis, and thus are not included as outcome measures in the current protocol. Furthermore, the enrollment numbers for the main study do not need to be increased, as the target sample size for the sub-study research will not be reached from this study alone, and we will only target recruiting as many possible within the target enrollment for the main study.

Description: A subset of 5-10 eumenorrheic (regularly cycling), pre-menopausal women not using hormonal birth control will undergo these optional additional procedures. Daily urine samples will collected over a complete menstrual cycle at baseline, and over 3 menstrual cycles at the beginning of the study (around months 1-3 of the intervention). Participants will be provided menstrual calendars to document overall menstrual cycle length during the weight loss intervention. During the urine collection period, they will be asked to collect daily, first void urine samples over the full menstrual cycle. Urine collection procedures will be based on those utilized by Dr. Santoro (co-mentor) in a large (n = 847) cohort study[38], and those currently being used in 17-0369. Specimen collection kits, including a supply of pre-labeled polypropylene tubes (pre-filled with glycerol to a final concentration of 7%), an indelible marker pen, disposable plastic cups, and storage boxes will be provided to participants. Women will be instructed to collect their first morning voided urine into a cup, fill two tubes to the indicated line (5 ml), and place each tub into a box in the freezer within 2 hours of collection. A specimen log will be provided to allow participants to record any irregularities in the collection, such as a failure to remember to freeze the tube within 2 hours. Women will be instructed to collect specimens from the 1st day of menstrual bleeding to the 1st day of bleeding. Weekly telephone calls, SMS texts, or emails will be performed to encourage adherence to the protocol and answer any questions that may arise. Urine samples will be assayed in Dr. Santoro's laboratory for progesterone and estrogen metabolites (pregnanediol 3-glucuronide [Pdg], and estrone conjugates [E1c]), and mid-cycle LH and FSH using a flurometric immunoassay (AutoDELFIA; Perkin Elmer & Centaur XP; Siemans). Urinary concentrations of Pdg and E1c are closely correlated with serum concentrations of estradiol and progesterone allowing for frequent,

minimally intrusive data collection. In addition, a tube of blood will be collected during the screening blood draw among these participants, and will be stored and assayed for Total Testosterone and Anti-Mullerian Hormone (AMH). This will allow us to examine predictors of obesity-related HPO axis suppression, and the effects of the behavioral weight loss intervention on HPO axis function distinct from the dysregulation of reproductive hormones associated with polycystic ovarian syndrome (PCOS). If, for whatever reason an additional tube of blood was not collected at a participants' screening visit, they will be asked to provide an additional blood draw in order to measure these assays. We will control for these hormone levels statistically, or potentially exclude data from participants whose labs are consistent with PCOS (AMH ≤ 6, serum Total Testosterone <100 ng/dL), whichever approach is deemed most appropriate.
Justification for Inclusion. Gonadotropins and ovarian hormones are considered key regulators of energy homeostasis and body composition among women, and are suppressed in response to energy restriction, which reflects an adaptive response to strategically shift energy away from reproduction and toward survival during resource scarcity. Given the limited success of current behavioral approaches to treat obesity in women, it is important to evaluate whether compensatory mechanisms have evolved to protect reproduction during times of reduced energy intake that make weight loss more difficult and explain some of the variability in weight loss response. Thus, we will evaluate whether changes in reproductive hormones impact weight loss response, as well as explore whether diet patterns or weight reduction may induce changes in levels of reproductive hormones in a subset of eumenorrheic, pre-menopausal women not using hormonal birth control.

Recruitment. Male and female enrollment will rely on recruitment strategies that have proven successful in attracting volunteers to other projects the mentor and applicant have conducted, including projects involving controlled feeding. The recruitment strategies will include:

-Fliers sent to households meeting specified demographics in geographic regions proximal to the University of Colorado (CU) Anschutz Medical Campus (AMC);

-E-mail bulletins – system-wide (e.g., CU AMC and University of Colorado Hospital [UCH]) e-mail blasts

-Suburban newspaper advertisements – there are many suburban journals in the Metro area that are issued on a weekly or monthly basis; we specifically advertise in those that target neighborhoods in relative proximity to CU AMC and those that target persons from under-represented minority groups;

-Major newspaper advertisements – the major publication in the Denver Metropolitan area has a Health and Fitness section that runs once per week; advertisements in these sections have targeted appropriate individuals for our previous studies;

-University advertisements – advertisements will be placed on a quarterly basis in the two CU AMC publications that target students/faculty/staff;

-Community lectures – the investigators participate in a CU AMC-sponsored health-related lecture series for the community;

-Participation in health fairs and walk-in health clinics;

Using these methods, the investigative team has previously been successful in recruiting study populations with demographics similar to the surrounding Denver metropolitan area.

Pre-screening: Information for the pre-screening of potential subjects during the initial phase of recruitment will be collected and managed using REDCap (Research Electronic Data Capture)

Survey. REDCap is a secure, HIPAA compliant web application designed to support data capture for research studies. Upon completion of REDCap and determination of qualification by research personnel, volunteer subjects will receive a detailed explanation of the procedures involved in the study at a consent appointment. Individuals who meet the eligibility criteria for the optional Reproductive Hormone Sub-study will be identified in the pre-screening by research personnel, and will be given a detailed explanation of the procedures and an opportunity to separately consent to the sub-study during the consent appointment.

Consent. The consent process is part of the first orientation session, which takes about 1hr. Volunteers are either sent a copy of the consent form in advance or, if necessary, are given one at the start of the orientation; time is allowed to fully review of the consent form. Volunteers then meet with a member of the research team to a) have the study and what is expected of them explained in detail; b) discuss their reasons and motivation for wanting to participate to determine whether they are realistic; c) discuss any practical problems (e.g., scheduling conflicts, vacations) that could interfere with participation; d) have their questions answered; and e) demonstrate their ability to provide informed consent by describing their understanding of the major study goals and what is expected of them if they choose to participate. To avoid coercion, the presentation will stress that, 1) all research participation is voluntary, 2) subjects may withdraw at any time, and 3) the decision to participate or not participate will not affect the subjects' medical care or any benefits to which the subject is entitled.

Compensation. Subjects will be compensated up to $400 for participation in the study. The compensation will be prorated as follows: Subjects will receive $100 for completing the behavioral weight loss program and performing assessments at the 12 week time point and $100 at the 6 month follow up visit. Subjects who choose to participate in the optional overnight PSG and nocturnal metabolism study will be compensated $100 per study day, or a total of $200. Subjects who choose to participate in the optional reproductive hormone sub-study will be compensated up to $305 for participation in the study. Subjects will receive $70/month of complete (>80%) daily urine collection, plus $25 for tracking and reporting menstrual cycle length with menstrual calendars. In addition, participants who agree to participate in the focus groups at the end of the intervention will be compensated for their time in the form of $50 Amazon gift cards.

Potential Benefits to Study Participation. The potential benefits to an individual participant in the study are not known. Subjects enrolled in this study will potentially benefit by learning information about their health status. Subjects will receive information on test results including body composition, blood glucose profile, sleep, exercise patterns, energy expenditure and metabolic rate and any of the study information they may be interested in. Subjects will also learn information on body weight regulation through their participation. Subjects will also receive some compensation. The potential benefits of the study to the adult population in general could be significant. The study is expected to lead to new data on the relationships between physiological variables relevant to weight regulation and weight regain following weight loss. This information will inform future strategies for the prevention of weight regain. In light of these potential benefits, the risks to subjects are considered acceptable.

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

Risks

Extended daily fasting: Subjects will be asked to fast prior to the screening visit for a blood draw and resting metabolic rate measurement. Due to the extra testing procedures, the fasting period may extend beyond a person's habitual overnight fast. In addition, subjects randomized to the TRF + RCD arm of the study will be asked to extend fasting duration to 14 hours/day for 12 weeks. Extended fasting may result in irritability, nausea, light-headedness.

DXA: The DXA (to assess body composition) procedure involves exposure to ionizing radiation. DXA measurements (3 time points during the study for each subject) involve a total effective

radiation exposure that is roughly equivalent to spending 4 days outside in Denver (<50 mrems), or about <1% of the annual allowable exposure for radiation workers. Having trained technician's conduct the DXA scans, thereby reducing the likelihood of needing repeat assessments, minimizes this risk.

Continuous glucose monitoring: The Libre Freestyle Pro CGM sensor measures and stores glucose readings when worn on the body. It initially comes in two parts: one part is in the Sensor Pack and the other part is in the Sensor Applicator. By following the instructions, the Sensor is applied on the back of the patient's upper arm. The Sensor has a small, flexible tip that is inserted just under the skin. The Sensor can be worn for up to 14 days (although a shorter 7 day measurement period will be used in the present study). In a clinical study conducted in 4 centers and including 72 adults, there were no device-related serious adverse events. Mild skin irritation, such as erythema, edema, rash, bleeding, itching, bruising, scaling skin, and induration were reported around the insertion site and adhesive area by a moderate frequency of subjects (26 out of 72 or 36%). Pain was mostly reported as none with only one reported instance of mild pain.

Venipuncture: Venipuncture will be performed to obtain the screening blood sample. Approximately 10 ml of blood will be removed by putting a needle into a forearm vein. This is the standard medical procedure for obtaining a sample for blood tests. Subjects may temporarily experience pain or discomfort when the needle goes into the vein, but this should subside once the needle is in the vein. A bruise may form at the site after the needle is removed.

Sleep measurement (PSG): Measurement of the electrocardiogram (ECG-heart tracing) may cause some skin irritation from the sensors. Measurement of the electroencephalogram (EEG-brain wave activity), Electroocculogram (EOG-eye movement activity) Electromyogram (EMG-muscle activity on the chin and legs), nasal-oral air thermister (breathing in and out of the nose & mouth), and respiratory bands placed over the chest and abdomen, may cause some minor discomfort and/or skin irritation due to the paste used to attach the sensors. In addition, the paste used to hold sensors to the scalp may leave a flaky residue for several days. The physiological recording device is electrically isolated and complies with hospital standards for electrical safety.

Whole room calorimeter: Claustrophobia or noisiness in the calorimeter may disturb volunteers. However, the new room calorimeter at UC is large (12' x 12') with a large picture window, and claustrophobia has never been reported. Furthermore, potential volunteers will be shown the calorimeter during the screening to familiarize them with the calorimeter.

Behavioral weight loss program: While exercise and diet recommendations are based on the best evidence and extensive investigator experience, some participants may experience adverse effects of either dietary change or exercise recommendations. Subjects are informed of this risk. We will minimize these risks through close monitoring by the study investigators.

Economic risks: Possibility of finding a previously undiagnosed medical condition during screening and not having insurance coverage for further evaluation and treatment, as well as time lost from work or studies. Minimized by ensuring subjects are in an appropriate economic position to participate

Psychological risks: may involve the stress of identifying a previous unknown medical condition during the pre-screening selection.

Confidentiality and privacy: The use of questionnaires, interviews, and collection of personal medical information poses a risk to confidentiality and privacy.

Optional Reproductive Hormones Sub-Study: The collection of cycle length and urine samples for assessment of reproductive hormones could identify a factor which predicts weight loss response and carries no known risk.

Protection against Risk

Risks of study related complications will be reduced by carefully selecting participants for participation. We have clearly defined the contraindications to participation. Every effort will be made to minimize risks including a review of participants' health before the testing. A baseline

biochemical profile (CBC, Chemistry panel including liver function tests, fasting glucose, TSH) along with blood pressure, heart rate, weight and height will be obtained at baseline. The study physician will contact the family physician, as needed, to clarify any issues that arise. The risk of finding a previously undiagnosed medical condition will be explained clearly as part of the informed consent, with the possibility that the condition(s) might require follow-up evaluation and treatment. This study will be scheduled to minimize time lost from work or studies, and subjects will receive some compensation for the time involved in completing the studies detailed herein.

Members of the Research team including the nursing staff on the CTRC are trained and experienced in all of the methods and measures that will be performed. These measures will be performed in a controlled clinical environment (the CTRC) with access to a full complement of hospital services. As discussed above, patients without significant co-morbidities will be recruited for this study to minimize the risks of serious adverse events.

Extended daily fasting: Participants will be allowed to eat food following the resting metabolic rate test performed at the screening visit. Participants will be encouraged to bring a snack to the visit, but the CTRC can provide a granola bar and juice. Subjects randomized to the TRF+RCD group will be informed of the potential risks and discomforts associated with extended daily fasting prior to starting the behavioral weight loss intervention. Tolerance to the TRF + RCD will be evaluated weekly during the behavioral weight loss program and support will be provided to help subjects manage hunger and cravings.

DXA: The risk of radiation exposure is minimized by having trained technicians administer the procedures, thereby reducing the likelihood of needing repeat assessments. Scans will not be performed in pregnant women. Urine pregnancy tests will be done and verified as negative before DXA scans are done on women of reproductive age.

Continuous glucose monitoring: Subjects will be informed that the CGM sensor may cause from minor skin discomfort and/or skin irritation.

Venipuncture and blood draws: Subjects will be asked not to donate blood or have blood drawn for any other studies (unless medically necessary) for prior to and following completion of this study to minimize risk of anemia. During venipuncture and catheter placement, standard sterile technique will be utilized and experienced phlebotomists and/or nursing staff will perform venipuncture and sampling catheter placement to minimize bleeding, thrombosis and infection at the puncture site.

Sleep measurement (PSG): Subjects will be informed that measurement of the electrocardiogram (ECG) and electroencephalogram (EEG), electromyogram (EMG), electrooculogram (EOG), nasal-oral air thermister and nasal cannula (breathing in and out of the nose & mouth/nasal pressure), respiratory bands placed over the chest and abdomen, may cause some minor discomfort and/or skin irritation due to the paste used to attach the electrodes. Subjects will be informed that the paste used to hold electrodes to the scalp may leave a flaky residue for several days. Aveno cream can be used to help treat skin irritation. Nasal-oral air thermister, nasal cannula and respiratory bands can be adjusted if uncomfortable.

Behavioral weight loss program: We will minimize risks through close monitoring by the study investigators and staff members conducting the weight loss program (weekly contact).

Psychological risks will be minimized by explaining this as being part of the informed consent and making sure that if a significant medical condition is diagnosed as part of the screening, every effort will be made to explain the condition to the subject, with recommendations for follow-up. The stress of physical procedures will be minimized by explaining each procedure as clearly as possible prior to and during the procedure, and having experienced staff perform the invasive procedures.

Data and Safety Monitoring Plan (DSMP)

The safety monitoring will be performed by the research team, with oversight by an independent local Safety Officer. This is an investigator-initiated study being conducted at a single site. The risks in this study are minimized by the use of extensive inclusion and exclusion criteria, and by close monitoring of the research subjects. In addition, the involved researchers are qualified and experienced in all of the study procedures. Nonetheless, since this study places the subjects at

more than minimal risk, we will utilize the services of a volunteer to serve as a Safety Officer at UC. The PIs, COMIRB, and Independent Study Monitors (Safety Officer and CTRC Research Subjects Advocates) will share the primary responsibility for monitoring study data and safety. The DSMP has been developed with the input from the Independent Monitors.

Roles and responsibilities of the Safety Officer (SO). The safety officer will periodically review safety data and judge whether the overall safety of the trial remains acceptable. Any recommendations to alter study conduct will be based on safety not efficacy; hence, there is no cost to the sample size, power, or final alpha level of the analysis. The SO will specifically review all serious adverse events (SAEs) and summary reports of all adverse events. The sponsor and/or applicant may also make recommendations to the SO for additional data review should a concern arise. The SO may recommend a new course of action for a specific treatment group or may suggest other appropriate courses of action to address general study safety issues that may arise. If warranted, the SO may recommend that the protocol be modified, suspended temporarily, or terminated permanently. The SO will meet in an open format with the study team every year to review the study progress.

The SO will review the following data: adverse events, recruitment and enrollment statistics, race and ethnicity statistics, disqualified and excluded individuals, study progress timeline, procedures for data quality control and safety data (e.g. screening labs, enrollment, inclusion and exclusion criteria, adverse events). The data will be "frozen" 2 to 4 weeks before the SO review and the data will be sent to the SO approximately 7 days before the SO review. Based on the review the SO may recommend alterations or changes to the protocol. Meeting minutes will be prepared by the PI of the project and approved by the SO. The minutes will be sent to: CTRC, COMIRB (annually), research team, and NIH (with annual progress report).

Defining and reporting serious adverse events (SAEs). We will utilize the Common Terminology Criteria for defining Adverse Events (CTCAE). Adverse events will be graded as mild (Grade 1), moderate (Grade 2), severe (Grade 3), or life-threatening (Grade 4), with specific parameters according to the organ system involved. We will promptly notify the IRB (within 5 days of the occurrence) when unexpected SAEs occur, defined as death (Grade 5), life threatening illness, hospitalization or prolongation of hospitalization, congenital anomaly/birth defects, and persistent/significant disability. The IRB requires that any SAE that is unexpected and related or possibly related to the research intervention be reported. SAEs that are unrelated to the research intervention do not have to be reported to the IRB. Risks that are described in the protocol and consent do not have to be reported promptly to the IRB unless they occur more frequently or are more serious than expected. One exception to this rule is in the case of a death. All deaths must be reported, whether or not the death was related to the research.

Withdrawals and terminations. All withdrawals and terminations will be reviewed by the researchers involved in this proposed study and I on a case-by-case basis. The data will be scrutinized separately to assessment for association of the interventions with a specific adverse outcome. An individual subject may be terminated from the study based on the criteria listed below. Any subject that is terminated from the study based on these criteria will be reported to the COMIRB during the annual continuing review.

- Request by the volunteer to leave the study
- Evidence of deliberate non-compliance
- Alcohol abuse; illicit drug abuse
- Development of a chronic condition (e.g. Grave's disease, hypothyroidism, rheumatoid arthritis, congestive cardiac failure, or neurological disorders such as MS or stroke) likely to impact upon metabolic variables, requiring medications likely to impact upon metabolic variables, or impair the ability of the subject to participate
- Development of an acute condition (e.g. myocardial infarction, major depression, accidents outside of the study resulting in physical impairments) likely to impact upon metabolic variables, requiring medications likely to impact upon metabolic variables, or impair the ability of the subject to participate Subjects will be reviewed on a case-by-case basis and will be reported to the Data Safety Officer.

E. Potential Scientific Problems:

Adherence to the TRF protocol: This will be the first study to our knowledge that will assess the feasibility of using TRF in the context of a reduced calorie diet for weight loss. We will objectively measure compliance to the protocol at baseline and 12 weeks using CGM and photographic time-stamped food logs but will otherwise not know whether subjects randomized to the TRF group were compliant to the protocol. To limit the extent of possible non-compliance to the TRF arm we decided on a shorter intervention for our first study. We are also using a high intensity behavioral support program that will meet weekly to discuss dealing with hunger/cravings or other challenges that might be associated with TRF. We will also ask participants to keep a log documenting daily feeding duration (first and last energy intake events) during all intervention weeks.

F. Data Analysis Plan:

Sample Size Justification:. We will aim to recruit 60 participants in two cohorts.. The sample size was determined for the primary outcome, change in body weight (kg) at 12 weeks. Assuming 20% attrition, we will be left with n=48, or n=24 subjects per group. To justify the sample size, we computed the detectable difference that we have 80% power for based on existing preliminary and published data. We will assume an alpha=0.05 and use two-sample t-tests. We assumed that the RCD group will have similar weight loss as has been shown previously with the standard Colorado Weigh curriculum, in which the mean ± standard deviation (SD) weight loss using an intent-to-treat analysis at 12 weeks is -4.1 ± 3.7kg[57]. There are no prior relevant data for 12-week weight loss using TRF in the context of a weight loss intervention. However, prior data from the Gill and Panda study[26] of time restricted feeding without a weight loss intervention showed a mean weight loss at 16 weeks of -3.3 kg with a SD=3.4 kg. Given that we are combining the RCD and TRF treatments, the standard deviation is likely to be between 3.4kg and the sum of the variation of the RCD and time restricted studies (SD of the sum = $\sqrt{3.7^2 + 3.4^2} = 5.0$ kg). In our calculation, we assumed for the time restricted treatment group (RCD+TRF) a SD in-between the above lower and upper bounds of SD at 4.2kg. With these SD's, we have 80% power to detect a difference in mean weight loss between the two treatment groups of 3.5kg. This is slightly larger than the Gill and Panda study, but well within the range of the confidence interval of their result. In addition, this calculation is quite conservative. We will have power of 80% to detect an even smaller difference in weight loss between the groups, because in the final analysis we will adjust for baseline weight, which reduces the SD's in each of the groups and allows us to have power to detect smaller differences. The intervention will be modified if we are unable to recruit 30 participants, retain >70% of participants, or perform outcome measures within a 2-week window of the indicated time points in >90% of participants.

Statistical Analysis: Efficacy Testing: Baseline demographic characteristics will be summarized by treatment groups using descriptive statistics. Any imbalance between groups will be examined using two-sample t-tests and Fisher's exact tests as appropriate. The primary analysis will use the ITT principle to test our study hypotheses under practical and realistic conditions when not all participants follow or complete the program. All randomized participants will be analyzed. A linear mixed effect model (LMM) will serve as the primary method to handle missing values. The normality assumptions will be evaluated and transformations used (e.g. square root and log) as appropriate. Change in weight is the primary outcome variable and others will be secondary outcomes. Consequently, no adjustment for multiple outcomes will be applied. P-values <0.05 will be considered significant. No interim analysis is planned. Any imbalance in baseline characteristics between groups that could potentially be a confounding factor for the outcome will be adjusted for in the statistical model (i.e., age, sex, level of PA achieved during intervention). Differences between groups in weight, fat mass, lean mass, MVPA, EI, sleep outcomes, and glucose variability will be compared using a LMM. Specifically, the test of time by group interaction will be used to assess efficacy. Pearson's correlations will be used to assess the relationship between sleep and nocturnal metabolism outcomes and longitudinal weight regain at 6 months. Variability of Major

Outcome Measures: Standard deviation and effect sizes will be determined based on the LMM model estimates to assist with planning the large-scale trial.

G. Summarize Knowledge to be Gained:

The importance of understanding mechanisms underlying the tendency for obese people to regain weight after they have lost weight cannot be overstated given the current epidemic of overweight and obesity and the prevalence of chronic diseases in the U.S. and other developed countries worldwide related to the clinical problem of obesity. Obesity is a major risk factor for many clinical conditions including type 2 diabetes, cardiovascular diseases, heart disease, hypertension, cancer, arthritis and can lead to premature death and disability. Another major problem posed by the overweight/obesity pandemic is the concomitant rise of a cluster of life-shortening risk factors referred to as metabolic syndrome. The metabolic syndrome represents a constellation of metabolic features including insulin-resistance, dyslipidemia, hypertension, and non-alcoholic fatty liver disease. While not regarded as a chronic disease, the metabolic syndrome increases risk of developing type 2 diabetes and cardiovascular disease. Altogether 133 million Americans - almost 1 out of every 2 adults - has at least one chronic illness, which results in 7 out 10 deaths every year. Medical costs associated with obesity were estimated at $147 billion; the medical costs for people who are obese were $1,429 higher than those of normal weight. In total, 75% of the US health care is spent on people with chronic conditions. Many people are able to lose weight but weight regain is almost universal following weight loss and is the biggest impediment to addressing the obesity epidemic. In this context, the proposed study has strong scientific, clinical, public-health and economic relevance.

Future Directions. Data will be used to optimize the RCD+TRF program delivery and to inform the planning of a grant proposal for a large-scale interventional trial to test the efficacy of RCD+TRF as compared to standard RCD with respect to body weight, fat mass, PA, metabolic outcomes, glucose variability and sleep. Further directions include the evaluation of specific time periods for restriction (e.g., earlier vs later during the day), and shorter vs longer duration of restriction. The role of baseline eating behaviors, especially chronotype, can be explored in future studies. Additionally, it would be of interest to evaluate the use of TRF after weight loss, during the weight maintenance period or in individuals with metabolic disease (i.e. T2DM, hyperlipidemia).

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