IRB-APPROVED PROTOCOL

Effectiveness of Early Time-Restricted Eating on Weight Loss, Fat Loss, and Cardiometabolic Risk Factors in Adults with Obesity

15. Background - in nontechnical, lay language

Summarize in 2-3 paragraphs past experimental and/or clinical findings leading to the design of this protocol. Include any relevant past or current research by the PI. For drug and device studies, summarize the previous results (i.e., Phase I/II or III studies).

Time-restricted feeding (TRF) is a novel type of intermittent fasting that involves eating within a daily period of 10 hours or less, followed by fasting for at least 14 hours daily. Nearly two dozen studies in rodents report that TRF reduces body weight. Moreover, multiple rodent studies report that TRF improves glycemic control, lowers insulin levels, increases insulin sensitivity, and improves beta-cell function—even when food intake is matched to the control group or no weight loss occurs.

Building on these findings, we recently conducted two of the first pilot studies of TRF in humans. In our first pilot study in overweight adults—which was only four days long—TRF increased 24-hour fat oxidation (fat burning); decreased mean 24-hour glucose levels (i.e., blood sugar); reduced the hunger hormone ghrelin; and reduced swings in hunger. In our second pilot study, five weeks of TRF improved insulin sensitivity and increased beta-cell responsiveness (both essential for good blood sugar control) in men with prediabetes, despite identical food intake in the control arm of our crossover trial. In addition, two pilot trials conducted by other investigators reported that even though people were not intentionally losing weight, TRF tended to lower their body fat levels and/or increase their muscle mass. In aggregate, this pilot data suggests that TRF should not only increase weight loss but should also improve fat loss, muscle mass retention, glycemic control, and blood pressure.

However, no one has yet tested whether TRF enhances fat loss while losing weight or whether TRF can improve weight loss success. We therefore propose to conduct this two-arm, randomized controlled trial to test whether TRF improves weight loss, fat loss, and cardiometabolic health in adults with obesity, relative to normal dieting.

16. Participants (Screening and Selection)

a. How many participants are to be enrolled at UAB (if other sites relying on UAB IRB, list the number for each site)? **About 86 (expected range is 75-95), in order to have 30 completers per group**

If multi-site study, total number at all sites/institutions: **N/A**

b. Describe the characteristics of anticipated or planned participants (if multiple groups, repeat list for each group).

Sex: **Male and female**

Race/Ethnicity: **All**

Age: **25-75**

Health status: **Have obesity (BMI 30-60 kg/m^2) but do not have diabetes or an unstable chronic disease**

c. From what population(s) will the participants be derived? **Participants will be recruited from the new patient population at the UAB Weight Loss Medicine Clinic.**

Describe your ability to obtain access to the proposed population that will allow recruitment of the necessary number of participants: **We will recruit participants directly from the UAB Weight Loss Medicine Clinic using medical records. Our Co-investigators, Drs. Amy Warriner and John Cleek, are the Co-Directors at the UAB Weight Loss Medicine Clinic. The UAB Weight Loss Clinic sees about 25 new patients per week, from which we aim to enroll 3 per week.**

d. Describe the inclusion/exclusion criteria:

***Inclusion Criteria*: (a) Are a new patient at the UAB Weight Loss Medicine Clinic; (b) Aged 25-75 years old; (c) BMI between 30-60 kg/m^2 (inclusive); (d) Weigh less than 450 lbs; and (e) Wake up regularly between 4-9 am on most days.**

***Exclusion Criteria*: (a) Diagnosed with diabetes, have an HbA1c of ≥6.5%, or are on diabetes medication; (b) On weight loss medication; (c) Addition of or withdrawal from a chronic medication within the past 10 weeks; (d) Clinically significant laboratory abnormality (e.g., abnormal hemoglobin levels); (e) Significant gastrointestinal disease, major gastrointestinal surgery, or gallstones; (f) Significant cardiovascular, renal, cardiac, liver, lung, adrenal, or nervous system disease that might compromise the participant's safety or data validity; (g) Evidence of cancer (other than non-melanoma skin cancer) within the last 5 years; (h) Unstable psychiatric, sleep, or circadian conditions (common conditions such as sleep apnea and depression are acceptable as long as they are stabilized and not rapidly worsening); (i) Lost or gained more than 5 lbs of weight in the past month; (j) Currently perform overnight shift work more than once per week on average; (k) Regularly eat within an <10-hour period each day; (l) Regularly eat dinner before 6 pm; (m) Traveled more than two time zones away in the two months prior to enrolling in the trial; (n) Will travel more than one time zone away during the study; and (o) Pregnant or breastfeeding.**

e. If participants will comprise more than one group or stratification, describe each group (e.g., treatment/intervention, placebo, controls, sham treatment) **and** provide the number of participants anticipated in each group. **There will be two groups: (1) Time-restricted feeding (TRF): eat between 7 am-3 pm or (2) Control group (eat over a 12-hour or longer period each day, but otherwise unrestricted). We are aiming to have about 30 completers in each group.**

f. Indicate which, if any, of the special populations listed below will be involved in the protocol. Include the Special Populations Review Form (SPRF) if indicated.

☐ Pregnant Women: Attach SPRF—Pregnant Women, Fetuses, Neonates/Nonviable Neonates

☐ Fetuses: Attach SPRF—Pregnant Women, Fetuses, Neonates/Nonviable Neonates

☐ Neonates/Nonviable Neonates: SPRF—Pregnant Women, Fetuses, Neonates/Nonviable Neonates

☐ Prisoners: Attach SPRF—Prisoners

☐ Minors (<18 years old): Attach SPRF—Minors

☒ Employees or students at institution where research conducted

☐ Persons who are temporarily decisionally impaired

☐ Persons who are permanently decisionally impaired

☐ Non-English Speakers

For each box checked, describe why the group is included **and** the additional protections provided to protect the rights and welfare of these participants who are vulnerable to coercion: **We will not exclude students or employees of UAB since many patients at the UAB Weight Loss Medicine Clinic may be UAB students and employees. To ensure that the study is in no way linked to the participant's employment or student activities, the following language is included in the informed consent and will be abided by: "If you are a UAB student or employee, taking part in this research is not a part of your UAB classwork or duties. You can refuse to enroll, or withdraw after enrolling at any time before the study is over, with no effect on your class standing, grades, or job at UAB. You will not be offered or receive any special consideration if you take part in this research."**

g. List any persons other than those directly involved in the protocol who will be at risk. If none, enter "None": **None**

h. Describe the recruitment process (e.g., medical record review, referrals, letter of invitation, existing patients) that will be used to seek potential participants (e.g., individuals, records, specimens). Research recruitment by non-treating physicians/staff may require completion of Partial Waiver of Authorization for Recruitment/Screening.

(1) *Recruitment*. The UAB Weight Loss Medicine Clinic already sends an email to all new patients after their first appointment at the clinic is booked. This email describes what to expect during their first appointment at the clinic. To this standard-of-care email, we will add a blurb at the end of the email to advertise the study. Interested patients will contact our research staff for more information and/or to pre-screen for the study. We will also contact patients with upcoming visits in the next couple of weeks by email and/or by phone to see whether they are interested in potentially participating in the study. To contact patients directly, clinic staff will email a list of names, phone numbers, and email addresses to the research coordinator and study staff, who will contact the patients to see if they are interested in participating. Finally, we may also advertise in the UAB Reporter. In all cases, individuals who are interested in participating in the study will be pre-screened by phone, as described below.

(2) *Pre-Screen*. Prior to their first medical appointment at the UAB Weight Loss Medicine Clinic, participants will be called by the research coordinator or trained research staff over the phone to determine whether they are interested in the study and/or meet key

eligibility criteria. A general overview of the study and its requirements will be given at this stage. The research coordinator will send the names of potentially interested and eligible individuals to the study physicians so that the physicians and staff know ahead of time which patients to discuss the trial with.

(3) *First Clinic Appointment*. Candidates will complete their first medical appointment at the UAB Weight Loss Medicine Clinic. The study physicians will review the lab results and medical history and discuss the study with each interested candidate to see if participating in the study is appropriate for him/her. Those who are interested and eligible will then complete the informed consent and screening process.

i. If you will use recruitment materials (e.g., advertisements, flyers, letters) to reach potential participants, attach a copy of each item. If not, identify the source (e.g., IRB Protocol Number for approved databases) from which you will recruit participants. **We have enclosed our recruitment email ads. We may also post a flyer at the UAB Weight Loss Medicine Clinic. The flyer that will be used is attached.**

j. Describe the screening process/procedures for potential participants.

Informed Consent Process. **Following the process described in 16(d), those who are interested and eligible will then complete the informed consent and screening process. The consent process will be conducted at the UAB Weight Loss Medicine Clinic (but may also be conducted in a private room in the Webb Building, in the case of patients who need more time to decide whether to participate). During this screening visit, the nature and requirements of the study will be explained in detail to the applicant. Applicants will be informed of all risks and benefits of participation, that their participation is voluntary and will not compromise their ability to receive regular services at the UAB Weight Loss Medicine Clinic, and that they may choose to end their participation in the study at any time. Individuals who wish to enroll in the study will be required to sign the written informed consent document in front of study staff; no waivers of informed consent will be granted. Once the informed consent is signed, eligibility criteria will be re-confirmed, given the inclusion and exclusion criteria.**

***Appointment Reminders*. During the screening process, we will ask participants whether they prefer to have appointment reminders sent to them by phone, email, or text message. We will explain that appointment reminders sent by email or text message will not be encrypted. Thereafter, participants will initial the consent form to document whether they want appointment reminders by email, text message, or by phone call, and we will send appointment reminders to each participant in the format that each person prefers. The appointment reminder subject line for emails will be "UAB Appointment Reminder," and the body of the email or text message will mention only the date, time, and location of the appointment and the name of the study.**

17. Protocol Procedures, Methods, and Duration - in nontechnical, lay language

a. Describe the procedures for all aspects of your protocol. Tell us what you are doing.

Adults with obesity will be randomized to follow one of two schedules while losing weight:

(1) TRF: Eat between 7 am and 3 pm (8-hour eating period)

(2) Control: Eat over a 12-hour or longer daily period, but otherwise unrestricted

Intervention. **Participants will receive the standard care of care for weight loss treatment at the UAB Weight Loss Medicine Clinic and additionally will be randomized to follow one of the above two eating schedules. The standard of care is to counsel participants to reduce their daily caloric intake by 500 kcal/day below their resting metabolic rate, along with behavioral counseling and recommendations for diet and exercise. We will counsel participants to exercise for a minimum of 15-30 minutes per day for 5 days per week at a fitness-appropriate level customized to the individual, based on their current exercise habits and mobility. Participants will be instructed to follow their assigned eating schedule at least 6 days per week for 14 weeks. All participants will be required to report their daily adherence to their eating schedule via an electronic survey (see "Daily Survey"). Participants will receive a link to the "Daily Survey" via an email that contains no PHI. The subject line will be "Daily Survey: Day #." When the participants click on the link, it will redirect them to the REDCap survey, which is hosted on the HIPAA-compliant REDCap platform. The surveys will ask participants to report their daily meal times; whether they thought they achieved their reduced calorie target on that day; and any reasons for non-adherence.**

Behavioral Support**. We will provide the following behavioral support:**

- ***Weekly Group Classes*** **(1 hour per session). Participants will be provided with weekly classes on weight loss strategies, eating behaviors, and exercise at the UAB Weight Loss Medicine Clinic. These classes are offered four times per week and may be attended either in-person or online. Participants will be asked to attend an average of three classes per month (~75% attendance rate).**

- ***One-on-One Weight Loss Counseling (30 minutes per session)*****. At the end of Weeks 0, 2, 6, and 10, we will provide participants with 30 minutes of weight loss counseling with a registered dietitian or trained member of our staff. With the exception of Week 0 (which will be at the Webb Building), these sessions will be held at the UAB Weight Loss Medicine Clinic. Participants do not need to pay anything for these sessions; they are a benefit of participating in this study, and they are designed to help participants lose more weight. During these sessions, we will provide weight loss counseling and encouragement, as well as check participants' body weight to track their progress. We will also formally query participants about adverse events during these sessions.**

- ***Monthly Check-Ins (10 minutes per session).*** **At the end of Weeks 4, 8, and 12, participants will visit clinic staff in the UAB Weight Loss Medicine Clinic, where we will check their body weight.**

Aims & Measurements. **All study measurements will be performed during Week 0 (baseline) and Week 14 (post-intervention). Aim 1 outcomes (weight loss, fat loss, changes in lean mass) will be measured using a scale and Dual X-ray Absorptiometry (DXA). Aim 2 outcomes include fasting glucose, fasting insulin, HbA1c, lipids, blood pressure, and heart rate. Aim 3 outcomes—food consumption, adherence, and feasibility—will be assessed through food photography, attrition rates, daily adherence surveys, questionnaires, and an exit interview.**

Ancillary Studies. **In addition, we will conduct four ancillary studies:**

1. **Validation of a meal timing questionnaire (Dr. Willett)**
2. **An ancillary study to determine how weight loss impacts nitrogen metabolism and bacteria in the mouth (Drs. Hunter and Patel)**
3. **An ancillary study to determine how weight loss and/or meal timing impacts the metabolism of oxalate, a compound involved in kidney stones (Dr. Wood)**
4. **An ancillary study to determine how weight loss and/or meal timing impacts sodium excretion in urine, blood pressure regulation, and kidney health (Drs. Pollock and Pollock)**

Month #	0	1				2				3				4	
Week #	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Weight Loss Program															
Daily surveys (electronic surveys)		■	■	■	■	■	■	■	■	■	■	■	■	■	■
Group classes (online or UAB Weight Loss Medicine Clinic)		■	■	■	■	■	■	■	■	■	■	■	■	■	■
One-on-one counseling (UAB Weight Loss Medicine Clinic)*			■				■				■				
Weight check-ins (UAB Weight Loss Medicine Clinic)*					■				■				■		
Food Photographs and Urine Collections**															
Meal timing questionnaire	■													■	
Food photographs (3 days)	■													■	
Urine collections (2 days)	■													■	
Testing (come to the Webb Building)															
Weight		■													■
Height		■													■
Waist Circumference		■													■
Body scan (DXA)		■													■
Blood draws		■													■
Blood pressure and heart rate		■													■
Tongue swab and saliva collection		■													■
Questionnaires		■													■
Weight loss counseling		■													
Exit Interview															■

** Weight will be measured every two weeks during all counseling sessions and weight check-ins*

*** These tests will be performed in the week prior to Week 0 and Week 14 Testing*

***Food Photography & Urine Collections*. Participants will take photographs of all food that they eat for 3 days and collect all their urine for 2 days in the week prior to the Week 0 and Week 14 testing.**

- ***Food Photography*: During Week 0 and Week 14, participants will take photographs of all foods that they eat for 3 days using an iPhone. This is known as the Remote Food Photography Method®. We will use these photographs to get a sense of participants' usual diet. Two of these days will be normal working days, while the third day will be a non-work day (or the equivalent). For each item of food or beverage (except for water) that participants consume, they will take a "before" photo (before they start eating) and an "after" photo (after they stop eating) using an iPhone app called SmartIntake. When using the SmartIntake® app, participants place a reference card next to their food and take photographs of the food before and after they eat.**

  The Remote Food Photography Method® (RFPM) and SmartIntake® smartphone app were developed by Corby Martin, Ph.D., H. Raymond Allen, Ph.D., and colleagues at the Pennington Biomedical Research Center. The app includes text message capability, allowing participants to record descriptions of their food as well as study staff to send reminders to participants. Participants may either use their own iPhone, or if they do not have an iPhone or do not wish to use theirs, they will be loaned a phone to use for the study.

  The food photographs and food descriptive text will be sent wirelessly by the app from the iPhone to a server at Pennington Biomedical Research Center, and the Pennington team will analyze the images to estimate food intake. The images will have no PHI and will not show the participants. To analyze the data, the process relies on a computer program that was built by H. Ray Allen, Ph.D. and team called the Food Photography Application©. The program allows the operator to identify a match for each food from the Food and Nutrient Database for Dietary Studies and other sources, such as manufacturer's information and Nutrition Fact Panels, to calculate calories and nutrients. Additionally, the Pennington staff use the program to estimate portion size by visually comparing participants' food images to images of foods with a known portion size (i.e., standard portion images). This process enables researchers to estimate the food selected at the start of the meal and the plate waste (i.e., the leftover food), which is used to calculate food and nutrient intakes by difference. The RFPM has been found to accurately measure the energy and nutrient intake of adults, with an error of only 3.7%, when used as prescribed.

  Before and after each round of photographs, we will also email participants a short meal timing questionnaire, which takes about 1 minute to complete.

- ***Urine Collection.* While participants are photographing their food, they will also collect all their urine on four separate occasions: two on consecutive days during Week 0 and two on consecutive days during Week 14. Participants will collect all**

of their urine (four 12-hour collections for each two day period) in containers that we provide. We will give participants written instructions describing how to collect their urine. Participants will bring the containers with urine with them when they come for Week 0 and Week 14 testing. The urine samples will be used to determine how weight loss and/or meal timing affects several biomarkers related to kidney stones (such as oxalate, citrate, sodium, potassium, creatinine, calcium, phosphorus, and uric acid) and blood pressure (such as sodium, potassium, chloride, endothelin-1, and exosomes, nitric oxide, and aldosterone). Analytes related to kidney health (such as albumin, KIM-1, nephrin, and oxidative stress markers) may also be explored. The primary endpoint of the urine collections for Dr. Wood's ancillary study is oxalate, while the secondary endpoints include citrate, sodium, potassium, creatinine, calcium, phosphorus, and uric acid. For Dr. Wood's ancillary study, urine analytes will be measured using a clinical analyzer and ion chromatography-mass spectrometry. For Drs. Pollock and Pollock's ancillary study, measurements will be performed as follows:

- **Electrolytes by ion selective electrodes**
- **Kidney (renal) injury markers and endothelin-1 by ELISA with spectrophotometer**
- **Exosome by ultracentrifugation and subsequent specific protein abundance quantification by Western blotting and/or mass spectrometry**
- **Nitric oxide by high-performance liquid chromatography (HPLC)**
- **Hydrogen peroxide by amplex red assay**
- **Other analytes by ELISA or other assays**

***Week 0 and Week 14 Testing*. For these tests, participants will need to come to the Webb Nutrition Sciences building in the morning while they are fasting (i.e., at habitual breakfast time but before breakfast). The testing will take about 1.25 – 1.5 hours and consist of the following:**

- ***Dual X-Ray Absorptiometry.* DXA scans will be performed to measure changes in fat-mass and fat-free mass. Scans will be performed by the Metabolism Core of the UAB Nutrition Obesity Research Center using an iDXA whole-body scanner (GE-Lunar Radiation Corporation, Madison, WI). Urine pregnancy testing will be performed prior to each scan and only non-pregnant individuals will be scanned. Scans will be analyzed using CoreScan software, and muscle mass will be estimated using published equations. Fat loss will be quantified as the percent of weight lost as fat; lean mass retention will be quantified similarly. *[Note to reader: Retrospectively, we realize the wording here is unclear. This was better explained on the ClinicalTrials.gov website, where we stated that the primary fat loss outcome was the percent of weight lost as fat (i.e., the ratio of fat loss:weight loss) and the secondary fat loss outcome was changes in absolute fat mass, changes in fat-free mass, and regional changes thereof).]***

- ***Blood Draws and Assays*. CCTS nurses/phlebotomists in the CSRP or CRU will draw blood. We will use the blood specimens to measure glucose (blood sugar), insulin (a hormone involved in blood sugar control), HbA1c (measures the risk of diabetes), nitrate and nitrite (for the ancillary study on mouth bacteria), and lipids. Pending future funding, we will also measure an array of proteins, metabolites, and RNAs that are mostly related to diabetes, metabolism, cardiovascular disease, cancer, and aging in the future. In total, we will draw no more than 3.4 tablespoons of blood each time. Serum processing will be performed by the Sample Processing and Analytic Nexus of the CCTS. Serum assays will be performed using UAB Cores.**

- ***Body Weight, Height, Waist Circumference, Blood Pressure, and Heart Rate*. These endpoints will be measured according to standard procedures.**

- ***Tongue Swabs and Saliva Collection*. Participants will be asked to refrain from using mouthwash for one week prior this procedure. About 200-300 µl of saliva that contains mouth bacteria will be collected from participants. The samples will be collected by passing a sterile swab (spatula) over the tongue while applying gentle pressure to collect saliva. We will also assess participants' mouthwash habits (see Mouthwash questionnaire). The data collected will be used as part of an ancillary study (lead by Drs. Hunter and Patel) to determine the effects of weight loss on nitrate metabolism and nitrate-reducing bacteria in the oral microbiome. Nitrate reductase activity will be assessed by measuring the conversion of nitrate to nitrite using HPLC separation coupled with UV-Visible light detection on an EICOM HPLC analyzer. Data will be adjusted for the number of bacteria, and bacterial profiles will be assessed using 16S rRNA sequencing.**

- ***Questionnaires:* Participants will fill out the following behavioral questionnaires:**
  - **Patient Health Questionnaire-9 (PHQ-9) to assess depression**
  - **Profile of Mood States (POMS) to assess anxiety and other mood states**
  - **Pittsburgh Sleep Quality Index (PSQI) to measure sleep quality**
  - **Munich Chronotype Questionnaire (MCQ) to measure sleep timing, duration, and chronotype**
  - **Appetite questionnaire to measure appetite**
  - **Dutch Eating Behaviors Questionnaire (DEBQ) to measure eating behaviors (emotional, external, and restrained eating)**
  - **Baecke Physical Activity Questionnaire to measure physical activity and exercise**
  - **Mouthwash Questionnaire**
  - **UCLA Loneliness Scale to also help assess the quality of social relationships**
  - **Social Life and Daily Habits Survey**

- ***Semi-Structured Exit Interviews.* We will also conduct semi-structured interviews to collect data on intervention feasibility. The semi-structured interview will**

query participants on the following themes: intervention acceptability and feasibility in terms of the impact of the meal timing schedules on participants' social, family, and professional activities; participants' satisfaction with the meal timing schedules and with the outcomes of the intervention; and participants' meal timing preferences, based on their experiences and strategies. The interview will also assess what factors facilitate or served as barriers to adherence. The interview will be performed only post-intervention. (These interviews will be recorded for later transcription; this is stated in the Informed Consent. Audio recordings will be password-protected and deleted within 12 months after the last manuscript from the study is published or the study closes.)

***Randomization*. Randomization will be stratified by sex, race, and physical activity level to the two groups in a 1:1 allocation using permuted blocks of size 2 and/or 4.**

***Main Study Statistical Analyses*. Analyses will be performed blinded and using two-sided tests with a Type I error rate of α=0.05. All data will initially be checked for missing values, cleaned, and inspected to determine the ranges, identify outliers, and check for concordance with parametric statistical assumptions (continuous variables only), including normality and homoscedasticity. Data will be analyzed using linear (or generalized linear) mixed models, including ANCOVA models. Data for endpoints that involve repeated measures will also control for baseline values as covariates, following procedures outlined by Allison [42]. These endpoints will also be tested for associations with other potential covariates, such as biological sex, race, BMI, age, and chronotype, and significant covariates will be included in our models. The fat loss and lean mass retention analyses will be performed per-protocol, while all analyses with repeated measures will be conducted using intention-to-treat analysis and will be followed by per-protocol sensitivity analyses. Categorical variables (from the surveys/interviews) will be analyzed using the chi-square test, or Fisher's exact test if the assumptions for the chi-square test do not hold. We will try to recollect any missing data, but that which remains may be treated using multiple imputation as appropriate.**

***Ancillary Studies Statistical Analyses*. (1) To validate the meal timing questionnaire, a daily food distribution score will be created by fitting a regression line using the time of day as the independent variable and the amount of food (calories for the dietary records) as the dependent variable. To evaluate the validity of the simple daily food timing questions, we will calculate the correlation between the daily food distribution score derived from the simple questions and the score derived from the food photographs (the gold standard). (2) Nitrogen metabolism and oral microbiome data will be analyzed using ANOVAs, regression analysis, and principal component analysis. (3) The impact of weight loss and/or meal timing on urinary oxalate excretion will be analyzed using paired t-tests and/or linear mixed models. Regression analyses may also be performed. (4) Data related to sodium excretion and kidney function will primarily be analyzed using ANOVA methods (or the equivalent nonparametric methods if the data are not normally**

distributed) and by regression analyses. Analyses may potentially be adjusted for relevant baseline covariates (ANCOVAs or linear mixed models).

b. What is the probable length of time required for the entire protocol (i.e., recruitment through data analysis to study closure)? **1 year**

c. What is the total amount of time each participant will be involved? **14 weeks**

d. If different phases are involved, what is the duration of each phase in which the participants will be involved? If no phases are involved, enter "None." **None**

e. Will an interview script or questionnaire be used? ☒Yes ☐No
If Yes, attach a copy.

f. Will participants incur any costs as a result of their participation? ☒Yes ☐No
If Yes, describe the reason for and amount of each foreseeable cost. **Participants will have to pay for their own transportation to and from UAB. This is stated in the informed consent.**

g. Will participants be compensated? ☒Yes ☐No
If Yes, complete i-v.
i. Type: (e.g., cash, check, gift card, merchandise): **UAB Weight Loss Medicine Clinic Membership (weekly group classes and monthly one-on-one counseling to promote weight loss; an $850 value)**
ii. Amount or Value: **$850**
iii. Method (e.g., mail, at visit): **At visit**
iv. Timing of Payments: (e.g., every visit, each month): **Weekly classes**
v. Maximum Amount of Compensation per Participant: **$850 value. UAB Weight Loss Medicine has a 1-year clinic membership option for patients, which gives patients access to weekly classes and monthly visits with a Registered Dietitian and/or Eating Behaviorist. These services are not usually covered by insurance, and the clinic membership "bundles" these services for patients at an overall cost savings. Study participants will be provided access to the Weight Loss Medicine Clinic Membership while they participate in the study. If a participant drops out of the study, the Weight Loss Medicine membership services will be terminated. If a participant completes the study, they will have continued Weight Loss Medicine clinic membership services for the remainder of the 12 months from the time that they enrolled in the study.**

18. Benefits

Describe the potential benefits of the research.
Potential benefits to both research participants and others include reducing their body weight; retaining more lean mass and losing more fat mass when they lose weight; and reducing their risk of diabetes and cardiovascular disease. Participants will also receive

free weight loss counseling. Additionally, research participants may expect benefits from learning about their health. All participants will be offered copies of test results from procedures performed during the study. In the case of an abnormal test result or other incidental finding, participants will be instructed to visit their physician or other appropriate health care provider. Finally, participants may benefit from better understanding nutritional treatment and weight loss strategies.

19. Risks - in nontechnical, lay language

a. List the known risks for participants as a result of participation in the research. This should not include the minimal risk of loss of confidentiality. However, it should include any physical, psychological, social, economic, and/or legal risks. If there is a greater than minimal risk of loss of confidentiality describe why this is so. Do not list risks associated with the standard-of-care procedures.

NOTE: Risks included here should be included in the consent form or information sheet, as applicable.

There is minimal risk of headaches and thirst due to the dietary intervention in a small fraction of participants. Feelings of hunger, hypoglycemia, dizziness, and weakness are also possible. Possible risks from exercise range from fatigue to minor musculoskeletal problems/injuries to, in very rare cases, cardiovascular events. Minor orthopedic problems are also possible, but we expect most are self-corrected with rest and standard first aid. The DXA procedure involves exposure to very low levels of radiation (x-rays); DXA scans will not be performed in pregnant women, which will be confirmed by pregnancy testing. With blood draws, there is the possibility of discomfort, pain, bruising, and bleeding where the needle is inserted. There is also a very small risk of infection at the site of the blood draw. The tongue swabs will likely involve mild discomfort and may cause a gag reflex. Lastly, there is a very small but unlikely risk of a loss of confidentiality.

b. Estimate the frequency, severity, and reversibility of each risk listed. **DXA, blood draws, and tongue swabs will occur only twice during the trial. The diet and exercise risks occur throughout the trial. All the expected risks are minimal, mild in severity, and are expected to be reversible.**

c. Is this a therapeutic study or intervention? ☒Yes ☐No

If Yes, complete i.-iii.

i. Describe the standard of care in the setting where the research will be conducted:

The standard of care at the clinic consists of initial one-on-one counseling with a registered dietitian to reduce caloric intake by 500 kcal/day below each person's resting metabolic rate. Patients are prescribed exercise according to their mobility and meal plans according to their needs and preferences, which are usually a variant of a low-calorie DASH/Mediterranean-styled diet. Following initial counseling, patients visit with their assigned physician or the nurse practitioner every 4-8 weeks and are encouraged (but not required) to attend weekly group classes on topics ranging from nutrition to exercise/activity.

ii. Describe any other alternative treatments or interventions: **Patients can always choose either (a) to lose weight on their own or (b) to follow the weight loss programs at the clinic but not to enroll in the trial.**

iii. Describe any withholding of, delay in, or washout period for standard of care or alternative treatment that participants may be currently using: **There will likely be a slight delay of up to 1-2 weeks in starting to lose weight, which is necessitated by needing to go through the informed consent process and completing the baseline testing.**

d. Do you foresee that participants might need additional medical or psychological resources as a result of the research procedures/interventions? ☐Yes ☒No

If Yes, describe the provisions that have been made to make these resources available.

_____

e. Do the benefits or knowledge to be gained outweigh the risks to participants?

☒Yes ☐No

If No, provide justification for performing the research: _____

20. Precautions/Minimization of Risks

a. Describe precautions that will be taken to avoid risks and the means for monitoring to detect risks.

***Dietary Intervention Protections*. To reduce the risk of increased thirst and headaches that are potentially associated with TRF, participants will be encouraged to drink water and stay hydrated.**

***Exercise Protections.* To minimize risk, participants will receive handouts with safety tips, including to increase their activity level gradually, focus on form (not weight), warm-up and cool down, take their time, and progress wisely. They will also be counseled during the one-on-one counseling sessions to adopt exercises that are appropriate to their current ability level.**

***DXA Protections*. The amount of radiation used for this procedure is very small. The radiation dose is equivalent to less than one to two days of exposure to ambient radiation that people are naturally exposed to in the environment. To reduce the risk, pregnant women will be excluded from participating in the trial. Moreover, pregnancy testing will be performed prior to each scan to ensure that scans are not performed on pregnant individuals.**

***Blood Draw Protections*. Trained nurses and phlebotomists will perform the blood draws, using standard sterile techniques.**

***Tongue Swab Protections*. Only trained personnel will perform the tongue swabs using sterile materials.**

***Adverse Events*. Participants will be instructed to report adverse events as they occur, as well as will be given emergency contact information. In addition, we will formally query all participants about adverse events once a month.**

***Handling Incidental Findings*. If any clinically abnormal result or illness is uncovered, or if participants exhibit signs of depression or mental illness, the affected participant will be notified and referred to his/her physician or an appropriate health professional for treatment. Participants will be offered copies of any tests or incidental findings.**

If the protocol involves drugs or devices skip Items 20.b. and 20.c. and go to Item 21. Instead include this information in the Drug Review Sheet or Device Review Sheet, as applicable.

b. If hazards occur to an individual participant, describe (i) the criteria that will be used to decide whether that participant should be removed from the protocol; (ii) the procedure for removing such participants when necessary to protect their rights and welfare; and (iii) any special procedures, precautions, or follow-up that will be used to ensure the safety of other currently enrolled participants.
This is a minimal risk weight loss trial. In a five-week trial, two out of eight participants (25%) reported increased thirst and headaches, while in a 4-day trial, no participants out of 11 reported any adverse events. Participants will be encouraged to stay hydrated. We do not expect any significant hazards. Nonetheless, should any hazards occur, Drs. Amy Warinner and/or John Cleek—who are physicians and Co-Directors at the UAB Weight Loss Medicine Clinic—will evaluate the situation and determine (i) what medical follow-up is necessary to ensure the participant's safety and (ii) whether there are any risks to participant safety or data validity that merit withdrawing the participant from the trial. Any withdrawals will be documented. Lastly, any cases of suicidal ideology or major depression with be directly referred to the social worker who evaluates such cases for the UAB Weight Loss Medicine Clinic and/or UAB clinical psychologist Gareth Dutton, who will serve as a back-up, in case the social worker is not available.

c. If hazards occur that might make the risks of participation outweigh the benefits for all participants, describe (i) the criteria that will be used to stop or end the entire protocol and (ii) any special procedures, precautions, or follow-up that will be used to ensure the safety of currently enrolled participants. **Drs. Warriner and Cleek will review adverse event data regularly. This is a minimal risk trial. However, should the risks outweigh the benefits, the trial will be stopped. The IRB may also be consulted to ensure the safety of the enrolled participants.**

21. Informed Consent

a. Do you plan to obtain informed consent for this protocol? ☒Yes ☐No

If Yes, complete the items below.

If No, complete and include the Waiver of Informed Consent or Waiver of Authorization and Informed Consent, as applicable.

b. Do you plan to document informed consent (obtain signatures) for this protocol? ☒Yes ☐No
If Yes, complete the items below.
If No, complete the items below **and** include the Waiver of Informed Consent Documentation.

c. How will consent be obtained? **An electronic informed consent document in PDF format will be presented on an iPad at the in-person screening visit. Individuals who prefer to read and sign a paper version of the consent document will be allowed to do so. Prior to signing the document, the nature and requirements of the study will be explained in detail to the applicant, and each applicant's comprehension of the study will be confirmed verbally. Ample time will be allotted for potential participants to ask questions and have them answered by study personnel. Individuals who wish to enroll in the study will be required to sign the written informed consent document prior to starting the study; no waivers of informed consent will be granted. Participants who sign the PDF form of the consent form will do so using a stylus. Each participant will be emailed a copy (or if they prefer, given a printed copy) of his/her signed consent form. Any informed consent documents that are locally stored on the iPad will be transferred off of the iPad within 24 hours. The iPads will be both encrypted and password-protected for security. When the iPads are not being used, they will be stored in locked cabinets in the Webb Building. The signed informed consent documents will be transferred off of the iPads within 24 hours and stored on encrypted and password-protected computers in the Webb Building.**

d. Who will conduct the consent interview? **The research coordinator, Dr. Jamshed, or Mr. Hanick. If none of the three are available, then Dr. Peterson may also conduct the interview.**

e. Who are the persons who will provide consent, permission, and/or assent? **Participants only**

f. What steps will be taken to minimize the possibility of coercion or undue influence?
If a participant is still interested and eligible at the completion of the phone screen, the study coordinator will email the consent form to the participant so that (s)he may review it before the in-person screen. Applicants will have ample opportunity to have all their questions answered and may discuss their participation in the trial with whomever they want. Also, applicants will be informed of all risks and benefits of participation, that their participation is voluntary and will not compromise their ability to receive regular services at the UAB Weight Loss Medicine Clinic, and that they may choose to end their participation in the study at any time.

g. What language will the prospective participant and the legally authorized representative understand? **English**

h. What language will be used to obtain consent? **English**

i. If any potential participants will be, or will have been, in a stressful, painful, or drugged condition before or during the consent process, describe the precautions proposed to overcome the effect of the condition on the consent process. If not, enter "None." **None**

j. If any protocol-specific instruments will be used in the consenting process, such as supplemental handouts, videos, or websites, describe these here and provide a copy of each. If not, enter "None." **We will give participants a handout summarizing the study visits, locations, and time commitment required.**

k. How long will participants have between the time they are told about the protocol and the time they must decide whether to enroll? If not 24 hours or more, describe the proposed time interval and why the 24-hour minimum is neither feasible nor practical. **We will aim to give participants at least 24 hours to decide. However, we expect that some participants will have less than 24 hours between receiving the consenting and their first appointment in the UAB Weight Loss Medicine Clinic. All participants may request and will be granted a couple more days to review the informed consent and decide to participate.**

PROTOCOL AMENDMENTS

After the study launched, the only significant protocol amendments were changes in study personnel, clarification of the length of the testing visits, and changes to the statistical analysis plan following a change in the study statistician. The latter is detailed in the “Statistical Analysis Plan.”