This supplement contains the following:

1. **Trial protocol**
2. **Statistical analysis plan**

1. Trial protocol

Intermittent fasting for the treatment of type 2 diabetes

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1.0 Project Summary/Abstract

Background: Innovative lifestyle strategies to treat type 2 diabetes (T2DM) are critically needed. At present, daily calorie restriction (CR) is the main diet prescribed to patients with T2DM for weight loss. However, many patients find it difficult to adhere to CR because calorie intake must be vigilantly monitored every day. In light of these problems with CR, another approach that *limits timing of food intake*, instead of number of calories consumed, has been developed. This diet is called **"time restricted eating" (TRE)** and involves confining the period of food intake to 6-8 h per day. TRE allows individuals to self-select foods and eat ad libitum during a large part of the day, which greatly increases compliance to these protocols. Two recent controlled trials show that TRE is effective for weight loss and improved glycemic control in patients with T2DM. While these studies offer promise, these trials are limited by short trial duration (1-3 months), and no comparison to CR. The present study will be the first longer-term trial (24-week) to compare the effect of TRE versus CR and controls for weight management and improved glycemic control in individuals with obesity and T2DM.

Hypotheses: Accordingly, this study will test the following hypotheses: **(Hyp1)** The TRE group will be more adherent with the intervention versus CR and controls, which will result in greater energy restriction and weight loss; **(Hyp2)** The TRE group will experience greater improvements in HbA1c and glycemic control, versus CR and controls; **(Hyp3)** Occurrences of adverse events will be minimal in all groups, and the use of oral hypoglycemic agents and insulin will decrease more by TRE versus CR and controls.

Methods: A 24-week randomized, controlled, parallel-arm trial will be implemented. Adults with obesity and T2DM (n = 75) will be randomized to 1 of 3 groups: (1) 8-h TRE (n = 25) ad libitum food intake from 12-8 pm, fasting from 8-12pm daily, (2) CR (n = 25), 25% energy restriction daily; or 3) control (n = 25), ad libitum eating.

Significance: This study will be the first to show that TRE can be implemented as an alternative to traditional dieting for long-term weight management and improved glycemic control. The American Diabetes Association (ADA) has now included intermittent fasting in their Nutrition Therapy Report. The report explicitly highlights the need for more well powered longer-term RCTs of intermittent fasting. The present trial will provide crucial clinical evidence to support the use fasting in the ADA guidelines.

2.0 Background/ Scientific rationale

Approximately 1 in 10 Americans have type 2 diabetes (T2DM).[1] It is estimated that by 2050, 1 in 3 Americans will have T2DM, if current trends continue.[1] In view of these alarming projections, innovative lifestyle strategies to treat T2DM are critically needed. **Calorie restriction (CR)** is generally encouraged as the first line of therapy to help people with T2DM achieve their weight management goals and glycemic targets.[2,3] However, many patients find it difficult to adhere to CR because calorie intake must be vigilantly monitored every day. As such, adherence to CR decreases after 1-2 months and continues to decline thereafter.[4-8]

Considering these problems with CR, another approach that limits the timing of food intake, instead of the number of calories consumed, has been developed. This diet is called **time restricted eating" (TRE)** and involves confining the period of food intake to 6-8 h per day. TRE allows individuals to self-select foods and eat ad libitum during a large part of the day, which greatly increases compliance to these protocols.[9-13] In two recent studies [10,11] performed by our lab, we show that 6-h and 8-h TRE reduces energy intake by 350-550 kcal/d, and lowers body weight by ~3% after 8-12 weeks.

Accumulating evidence suggests that intermittent fasting may be more effective than CR for improving glycemic control. In a recent trial [14] that compared the effects fasting versus daily CR, insulin resistance decreased to a much greater extent in the fasting group (-53%) versus the daily CR group (-17%). Another trial reported dramatic increases in insulin sensitivity and beta-cell responsiveness with 6-h TRE, even in the absence of weight loss.[15]

Only two trials to date have examined the effects of TRE in patients with T2DM. In a 3-week cross-over trial by Andriessen and colleagues,[16] 9-h TRE decreased body weight by 1.1%, and increased time spent in the euglycemic range in 14 adults with T2DM, versus controls. Complementary to these findings, Che et al [17] showed that 12 weeks of 10-h TRE lowered body weight by 3.5% and decreased HbA1c by 1.5% in 120 adults with obesity and T2DM, versus controls. While these studies offer promise for the use of TRE in patients with diabetes, these trials are limited by short duration (3-12 weeks) and lack of comparison to standard care (i.e., daily CR).

The present study will be the first longer-term trial (24-week) to compare the effect of TRE versus CR and controls for weight management and improved glycemic control in individuals with obesity and T2DM.

3.0 Objective/Aims

Specific aim 1: <u>Body weight, energy restriction, and dietary adherence</u>
Hypothesis 1: Men and women with obesity and T2DM will be more adherent with the TRE intervention versus CR and controls, which will result in greater energy restriction and weight loss by week 24.

Specific aim 2: <u>HbA1c, glycemic control, oxidative stress, and cardiometabolic risk factors</u>
Hypothesis 2: Men and women with obesity and T2DM will experience greater improvements in HbA1c, glycemic control variables (by CGM), by TRE versus CR and controls, due to greater reductions in body weight by week 24.

Specific aim 3: <u>Medication use and occurrence of adverse events</u>
Hypothesis 3: Use of oral hypoglycemic agents and insulin will decrease more by TRE versus CR and controls by week 24. Occurrences of adverse events (i.e. glycemic, gastrointestinal, neurological events) will be minimal in the TRE, CR and controls groups, with no differences between groups.

4.0 Eligibility

Adults with obesity and T2DM will be recruited by flyers posted around the University of Illinois at Chicago (UIC) campus and on social media. Subject eligibility will be assessed and determined by Vicky Pavlou M.S., R.D., or key research personnel identified in Appendix P. Screening will be conducted at the **Human Nutrition Research Unit (HNRU)** located in the Applied Health Sciences Building (1919 West Taylor St, Room 121C).

4.1 Inclusion criteria:

- Age between 18 to 80 years old
- BMI between 30 and 50 kg/m^2
- Previously diagnosed with T2DM
- Have an HbA1c level between 6.5-11%
- Are sedentary or lightly active [18]

4.2 Exclusion criteria:

- Are normoglycemic (HbA1c <5.7%), have prediabetes: HbA1c: 5.7-6.4%, or HbA1c above 11%
- Have a history of eating disorders (anorexia, bulimia, or binge eating disorder)
- Are not weight stable for 3 months prior to the beginning of study (weight gain or loss > 4%)
- Are not able to keep a food diary for 7 consecutive days during screening
- Are eating within less than a 10-hour window at baseline
- Perimenopausal (menses does not appear every 27-32d)
- Are pregnant, or trying to become pregnant
- Are night shift workers
- Are current smokers

5.0 Subject enrollment

Independently living subjects from the Chicago area will be recruited by flyers posted around the University of Illinois at Chicago (UIC) campus. The flyer will also be posted to social media outlets including: Instagram, Facebook, and Twitter. ResearchMatch.org will also be used to recruit participants. All study procedures will be conducted at the Human Nutrition Research Unit (HNRU) located in the Applied Health Sciences Building (1919 West Taylor St, Room 121C).

Each subject will attend 2 screening visits. During **Visit 1** subjects will be screened by the Study Manager, Vicky Pavlou MS, RD, via questionnaire, which will assess eligibility based on the requirements listed above. The following parameters will also be assessed: body weight and height (for BMI), pregnancy test, and a blood test (for HbA1c). The Study Manager will also distribute a 7-day food record and provide detailed instructions on how to complete the records. **Visit 2** will be scheduled 10 days after the first screening. During this visit, the food record will be assessed for adequacy (to evaluate each participant's motivation to participate in the study and their baseline eating window). Consent will be obtained prior to the administration of the screening questionnaire by a member of the study team (key research personnel identified in Appendix P).

Subjects who meet all the inclusion/exclusion criteria will be invited to participate in the study. Eligible subjects will be randomized by way of a stratified random sample. The sample frame will be divided into strata based on BMI, sex, age, and HbA1c. Subjects from each stratum will then be randomized to 1 of 3 groups: 1) TRE, 2) CR, 3) control.

The consent forms and screening questionnaires from screen failures will be kept in a locked filing cabinet until the study is over. To minimize the possibility of coercion, only subjects who respond to the study ad will be called in for screening. If they meet all the inclusion/exclusion criteria they will be invited to participate in the study. All the subjects will have the right to withdraw from the study at any time.

6.0 Study design and procedures

A 24-week randomized, controlled, parallel-arm trial will be implemented. Subjects with obesity and T2DM (n = 75) will be randomized to 1 of 3 groups: (1) TRE (n = 25) ad libitum food intake from 12 pm to 8 pm, fasting (with zero-calorie beverages) from 8 pm to 12 pm daily, (2) CR (n = 25), 25% energy restriction every day; or 3) control (n = 25), ad libitum food intake daily and eating within >10-h window each day. All in-person study activities will be carried out at the Human Nutrition Research Unit (HNRU) located in the Applied Health Sciences Building (1919 West Taylor St, Room 121C). A total of 4 blood samples (20 ml each) will be collected throughout the trial.

Diet interventions

Group	24-week intervention protocol
TRE	8-h eating window Ad libitum food intake from 12-8 pm every day Fasting from 8-12 pm every day (16-h fast)
CR	25% energy restriction every day
Control	Ad libitum food intake, eating within >10 h window/d

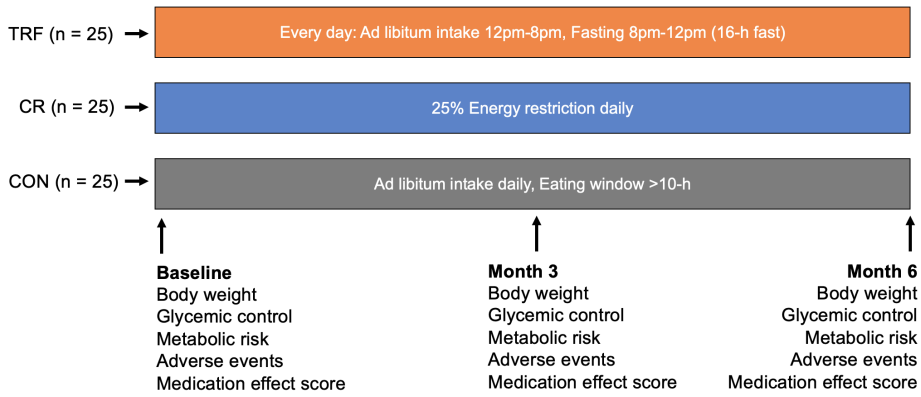
- **TRE weight loss diet (8-h eating window):** The TRE group will be instructed to eat ad libitum from 12-8pm daily, and fast from 8-12 pm (16-h fast). During the 8-h eating window, there will be no restrictions on types or quantities of foods consumed. Moreover, participants will not be required to monitor caloric intake during this ad libitum feeding period. During the fasting period, participants will be encouraged to drink plenty of water and will be permitted to consume energy-free drinks, such as black tea, coffee, and diet sodas (limit 2 diet sodas/d, since these beverages may increase sugar craving [19]). TRE participants will meet with the dietician every week (by phone or zoom) to review their diet adherence, medication changes and adverse events from months 0-3, then bi-weekly from months 4-6. Subjects will also be taught how to make healthy food choices that conform to ADA nutrition guidelines for people with T2DM.[2]
- **CR weight loss diet:** CR subjects will restrict energy intake by 25% of their baseline energy needs every day. CR subjects will meet with the study dietician during the first week of the study (by phone or zoom) to develop individualized weight loss meal plans to help them meet their daily calorie goal for weight loss. The weight loss meal plans will include menus, portion sizes, and food lists that are consistent with the subject's food preferences and prescribed calorie levels for weight loss. Participants will meet with the dietician every week (by phone or zoom) to review and modify the meal plans from months 0-3, then bi-weekly from months 4-6. Diet adherence, medication changes, and adverse events will also be monitored during these calls. During these sessions, subjects will also be instructed how to make general healthy food choices that conform to ADA nutrition therapy guidelines for people with T2DM.[2]

- **Control group protocol:** Controls will be instructed to maintain their weight throughout the entire trial, and not to change eating or physical activity habits. Controls will not receive dietary counselling. The study manager will meet with the controls every week (by phone or zoom) to ensure that they are staying weight stable and not altering their diet. Medication changes and adverse events will also be evaluated during these calls.

  Justification for inclusion of control group: The key purpose of including a control group in our design is to make the study scientifically robust. The control group will help us to assess changes in key outcome measures (HbA1c and body weight) that occur because of the interventions (TRE and CR), versus the control condition (i.e. what patients regularly do to manage their T2DM).

Calorie restriction is a standard diet used for the management of T2DM. [20] However, many patients with T2DM struggle with adhering to calorie reduced diets. [21,22] It is anticipated that most of the participants screened will not be following a calorie reduced diet. In addition, we will be excluding participants who are not weight stable for 3 months prior to the beginning of the trial (see exclusion criteria). This will help ensure that none of the participants are following a CR diet at baseline.

Summary of intervention groups and key outcome measures



Medication management and adverse event monitoring by the study Endocrinologist: Dr. Terry Unterman, MD and Julienne Sanchez, MD (UIC Diabetes Center & Endocrinology Clinic), will serve as the on-site study endocrinologists. The medication management protocol was developed after reviewing the literature [23,24] and consulting with Dr. Unterman and other endocrinologists. Management will occur in conjunction with the participant, the study dietitian, the study endocrinologist, as well as with the participant's medical practitioner (if different from the study endocrinologist).

Blood glucose monitoring: All participants will wear a continuous glucose monitor (CGM) (Dexcom) for 10 days at baseline, week 12 and 24. CGM data will be used to detect hypoglycemic events (glucose level <70 mg/dl) or hyperglycemic events (glucose level >180 mg/dl). When subjects are not wearing the CGM, they will test and record their blood glucose levels daily before the first meal of the day, before the largest meal of the day (typically dinner), and 2 hours after the largest meal of the day (with a lancing device and glucose monitor). If blood glucose is below 70 mg/dl, the subject will be asked to contact the study coordinator. Medication changes will be made over the phone or by email in consultation with the study endocrinologist or the participant's endocrinologist. If glucose levels are above 180 mg/dl, dietary compliance will be checked, and medication changes will be made if necessary.

Medication management protocol

TRE and CR participants: If the baseline HbA1c level is below 7%, the protocol will require the discontinuation of sulfonylureas, the dose of short-acting insulin will be reduced by 50%, and long-acting will remain unchanged. If the HbA1c level is greater than 7% but below 8.5%, the dose of sulfonylureas will be reduced by 50%, short-acting insulin will be reduced by 10%, and long-acting will remain unchanged. If the HbA1c level is greater than 8.5%, sulfonylurea medications, short-acting insulin and long-acting insulin will remain unchanged. The endocrinologist will work with each participant individually to ensure the best care.

Control participants: Since the control subjects will not be making any major changes to their diet, and will experience minimal weight change, we do not foresee them having to make any changes to their medications.

HbA1c	OHA (Sulfonylureas)	Short-acting insulin	Long-acting insulin
<7%	TRE and CR: Discontinue.	TRE and CR: Reduce dose by 50%	TRE and CR: Continues
7-8.5%	TRE and CR: Reduce dose by 50%	TRE and CR: Reduce dose by 10%	TRE and CR: Continues
>8.5%	TRE and CR: Continues	TRE and CR: Continues	TRE and CR: Continues

Study activities

Study activities		Study duration – 24 weeks																							
	Screen	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Informed consent	•																									
Screening questionnaire	•																									
Pregnancy test	•	•												•												•
Body weight	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Waist circumference		•												•												•
Diet adherence		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Blood pressure/heart rate		•												•												•
DXA		•												•												•
Blood draw	•													•												•
Activity monitor (7-days)		•												•												•
Continuous glucose monitor		•												•												•
Food record (ASA-24)		•												•												•
Sleep questionnaires		•												•												•
Appetite questionnaires		•												•												•
Quality of life/mood		•												•												•
Eating disorders		•												•												•
Adverse event survey		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Medication effect score		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

***Note: The only in-person visits will be held at screening visit 1 and 2, week 0, 12, 24 (5 in-person visits total over 24-weeks). All other visits will be held over the phone/zoom. Body weight will be measured by the participant at home and reported to the study coordinator over the phone/zoom.**

Measurement of outcomes:

Body weight, blood pressure, heart rate: Body weight measurements will be taken weekly, upon waking, in the fasted state at home. Body weight scales will be distributed to each subject at baseline (Withings Smart Scale). Measurements will be taken in light clothing and without shoes using a WiFi-enabled electronic scale (data from the scale is sent directly to the study coordinator). Height will be assessed using a wall-mounted stadiometer at screening. BMI will be assessed as kg/m^2. Blood pressure and heart rate will be assessed using a blood pressure cuff after a 10-minute rest.

Body composition: Dual energy X-ray absorptiometry (DXA) will be performed on all subjects at week 0, 12, 24 (iDXA, GE Inc) to assess fat mass, fat free mass, and visceral fat mass. Waist circumference will be measured midway between the lowest ribs and the iliac crest.

Blood sampling: Twelve-hour fasting blood samples will be collected at screening (visit 1) and during the study at week 0, 12, 24 (20 ml at each time point). All blood draws will be performed at the HNRU by Vicky Pavlou, MS, RD. Blood will be centrifuged for 15 min at 520 x g and 4°C to separate plasma from red cells, and will be stored at -80°C at the HNRU.

Activity monitor: All subjects will be instructed to maintain their regular daily activity habits (i.e not join a gym or participate in other exercise classes). Alterations in physical activity habits will be quantified by an activity monitor (Fitbit Alta HR)) that will be worn on the wrist for 7 days at week 0,12, 24.

Food record: Dietary intake will be assessed in all groups by a 7-day food recall using the ASA-24 website at week 0,12, 24. Subjects will be given detailed guidelines on how to complete the records by the dietician at baseline.

TRE and CR adherence: Adherence to the TRE window will be measured using a daily adherence log (or Redcap app), which will record the times each participant starts and stops eating each day. If the log indicates that the participant ate within the +/- 1 h of the prescribed window, that day will be labeled "adherent". If the log indicates that the participant consumed food outside of the prescribed window, that day will be labeled as "non-adherent". Adherence to the TRE diet will be assessed as the number of adherent days per week. CR participants will be considered "adherent" when their actual energy intake, determined via food records, is within 200 kcal of their prescribed daily energy goal. In addition, TRE and CR participants will be asked to complete a survey at the end of the study asking: "How difficult was it to adhere to the diet?" Participants will be able to select one of four categories "Easy," "Moderately Easy," "Moderately Difficult," or "Difficult." The proportion of subjects in selecting each category will then be calculated.

Metabolic disease risk factors: Plasma metabolic disease risk factors will be assessed at baseline week 0, 12, 24. Plasma total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride concentrations will be measured by a commercial lab (Medstar, IN). HbA1c will also be assessed by a commercial lab (Medstar, IN). Fasting plasma glucose concentrations will be measured with a hexokinase reagent kit in duplicate (A-gent glucose test, Abbott, South Pasadena, CA). Fasting insulin will be measured as total immunoreactive insulin (Coat-A-Count Insulin, Los Angeles, CA). Complete blood count (CBC) will be assessed by a commercial lab (Medstar, IL). Insulin sensitivity will be calculated by the QUICKI formula: [1/(log [fasting insulin in mU/l] + log [fasting glucose in mg/dl]). Insulin resistance (IR) will be calculated by the HOMA (Homeostasis Model Assessment) method: [HOMA-IR = Fasting insulin (μlU/ml) × Fasting glucose (mg/dL) / 405].

Continuous glucose monitor: Participants will wear a continuous glucose monitor (CGM; Dexcom) over 10 days at week 0, 12, and week 24 to assess changes in glucose levels. CGM data will be used to compute overall percent of total time spent in euglycemic range (70-180 mg/dl) and mean glucose levels. The sensor will be placed on the backside of the upper arm by the study manager, Vicky Pavlou, MS, RD. The sensor

will be removed by the subject at home according to the manufacturer's instructions. The CGM will not be returned to the research center until the next study visit.

Medication effect score (MES): Medication dosages will be recorded weekly, and the medication effect score (MES) will be used to quantify changes. The MES is calculated as (actual drug dose/maximum drug dose) × drug mean adjustment factor. [25] A higher MES corresponded to a higher dose of diabetes medication, and a reduction in MES corresponded to a reduction in diabetes medication. For example, a 0.5-MES change in the dose of metformin hydrochloride equals a reduction by 1000 mg. [25]

Adverse event questionnaire: Occurrence of adverse events will be recorded each week by an adverse events survey. Measures will include: Neurological issues (dizziness, headache, fatigue, irritability), gastrointestinal issues (nausea, diarrhea, constipation, dry mouth), as well as episodes of diabetic ketoacidosis, hypoglycemia and hyperglycemia (assessed by glucose monitor daily).

Inflammation and oxidative stress: Inflammatory and oxidative stress markers will be measured at week 0, 12, 24. C-reactive protein (CRP) will be measured in duplicate using Immulite 1000 High Sensitivity CRP kits (Diagnostic Products Corpor- ation, Los Angeles, CA). ELISA will be used to measure adiponectin, leptin, TNF-a, and IL-6, 8-isoprostane (Caymen Chemical, Anne Arbor, MI), 4-hydroxynonenal adducts, protein carbonyls, and nitrotyrosine (Cell BioLabs, San Diego, CA).

Appetite assessment: Appetite will be assessed at week 0, 12, 24. An appetite questionnaire will be used to assess hunger and fullness over the course of 2 hours (in response to a test meal). Appetite ratings will be taken at these time points: -15 min (15 min before consuming the meal), 0 min (right after consuming the meal), and at 30, 90, and 120 min after the meal. The appetite questionnaire is attached to the submission.

Sleep, mood, quality of life, readiness for change, eating disorder, demographics questionnaires: Appetite, sleep, mood, quality of life, readiness for change, eating disorder symptoms, and demographics will be assessed by validated questionnaires at week 0, 12, 24.

7.0 Expected risk and benefits

Risks:

1. Blood draws: Blood drawing may cause temporary discomfort from the needle stick, bruising, and minor infection. A total of 4 blood samples (20 ml each) will be collected at these time points:

- Screening visit 1 = 1 blood draw (20 ml)
- week 0 = 1 blood draw (20 ml)
- week 12 = 1 blood draw (20 ml)
- week 24 = 1 blood draw (20 ml)

2. Risk of hypoglycemia during TRE: We understand that fasting for 16-h/d during TRE may increase the risk of hypoglycemia. To lower the risk of hypoglycemia, participants will be asked to test and record their fasting blood glucose levels daily before the first meal of the day, before the largest meal of the day (typically dinner), and 2 hours after the largest meal of the day. If the blood glucose level is less than 70 mg/dl, the participant will be asked to contact the study coordinator and the study endocrinologist. The study coordinator will be on call 24h per day. Medication changes will then made over the telephone or via email in consultation with the study endocrinologist.

3. DXA scans: DXA scans will be performed to assess body composition (3 scans total). Subjects who participate in this study will receive a small amount of radiation from the DXA scanning. The amount is similar to that received in many standard x-ray procedures, but is considerably more than subjects would receive from natural daily exposure or in the normal course of treatment, and it carries at least a theoretical risk.

4. Continuous glucose monitors: These devices are widely used and are generally safe. Insertion of the sensor may cause temporary discomfort from the needle stick, bruising, and infection. Insertion and

removal of the sensor will be performed according to the manufacturer's instructions (manual attached to submission).

5. Loss of confidentiality: Loss of confidentiality is also a potential risk. To protect subject identity, only code numbers (i.e. randomly generated numbers) will identify all laboratory specimens, evaluation forms, reports, and other records.

6. Questionnaires: Subjects may also experience psychological discomfort while answering the questions posed in the questionnaires. If this is the case, the subject will be encouraged to speak with the study coordinator, and answer only the questions that they feel comfortable answering.

Benefits: Subjects may lose weight and improve their HbA1c level as a result of treatment. Since weight loss has been shown to improve some metabolic disease risk factors, subjects partaking in the treatment may experience these benefits to their overall health. No benefits can be guaranteed, however.

8.0 Data collection and management procedures

Sources of data include body weight and body composition measurements, blood pressure measurements, biochemical analyses of blood draws, continuous glucose monitor measurements, activity monitor measurements, and questionnaire responses. Questionnaires will be stored in locked filing cabinets at the HNRU. To protect subject identity, code numbers only will identify all laboratory specimens, evaluation forms, reports, and other records. Subject identities will not be used in any reports or publications resulting from this study. All patient records will be kept in locked files; code sheets linking the patient's name to a patient identification number will be stored separately in a locked file cabinet. Only study personnel will have access to the files. Blood specimens and stool samples will be stored at the HNRU in a locked -80C freezer. Specimens will not be labeled with any personal identifiers. Only the subject ID and study week will be on the label of the blood specimens. Code sheets linking the patient's name to a patient identification number will be stored separately in a locked file cabinet.

REDCap: REDCap will be used to store and collect data for this study. The only personnel that will have access to REDCap are: Vicky Pavlou, Sofia Cienfuegos, Shuhao Lin, Kelsey Gabel, and Krista Varady (all identified in Appendix P). **Protections in place to maintain confidentiality in REDCap:** The data will be stored with coded/indirect identifiers only. Subject identities will not be used in any surveys or spreadsheets in REDCap. Code sheets linking the patient's name to a patient identification number will be stored separately in a locked file cabinet in the HNRU. Only study personnel will have access to the files. Access to the study's REDCap records will require password access.

9.0 Data analysis

Data will be analyzed by Shaina Alexandria and Krista Varady by SPSS software (SPSS v24).

10.0 Quality control and quality assurance

The PI will be responsible for the evaluation of data quality, and this will be done on a weekly basis by evaluating biochemical analyses output and reviewing adverse event questionnaire responses.

11.0 Data and safety monitoring

Assessment of Risk: The risk of significant adverse events to participants in this study is low. During the 24-week study, participants will undergo 4 blood draws, 3 DXA scans, 3 x 10 days of continuous glucose monitoring, and TRE.

Anticipated Adverse Events: Blood drawing may cause temporary discomfort, bleeding or bruising at the needle site and in rare cases fainting and infection. As for the DXA scan, the radiation dose associated with DXA measurements is very low or even insignificant in comparison with background radiation levels. Continuous glucose monitors are widely used and are generally safe. Insertion of the sensor may cause temporary discomfort from the needle stick, bruising, and infection. Loss of confidentiality may also be a risk. Fasting for 16-h/d during TRE may increase the risk of hypoglycemia. To lower the risk of hypoglycemia, subjects will test fasting blood glucose daily before the first meal of the day, in the middle of the day, and before bed. If the blood glucose level is less than 70 mg/dl, the subjects will contact the study coordinator (who will be on call 24h/d). Medication changes will then made over the telephone or via email in consultation with the subject's endocrinologist.

Adverse Event Grading Scale: Because only minor adverse events related to blood drawing, DXA scanning, continuous glucose monitoring, and TRE may occur, no grading scale is necessary.

Reporting of Adverse Events: Reporting of adverse events will follow requirements mandated by the University of Illinois Office for Protection of Research Subjects (OPRS). The OPRS will receive a verbal or e-mail report of any serious adverse event (SAE) that occurs during the conduct of the study within 48 hours. This will be followed by a detailed written report within 10 working days. In addition, any incidents or problems involving the conduct of the study or patient participation, including problems with the recruitment and/or consent processes will be reported within 10 working days.

Safety Monitoring Plan: Sterile procedures will be employed for all blood drawing and continuous glucose monitoring procedures. Subjects will be provided with a 24-hour contact phone number to report any persistent problems. The OPRS will be notified of any adverse events as described above.

Frequency of Safety Reviews: Safety reviews will be conducted annually in conjunction with the protocol renewal to OPRS.

Persons to Perform Safety Reviews: The PI will be responsible for reporting any adverse events and conducting the annual safety review of study data.

12.0 Statistical considerations

For the sample size calculation, we estimated that TRE and CR groups would reduce body weight by 7% and 3% (extrapolated from our pilot data), respectively, by month 6, versus controls (no change in body weight). We calculated that n = 21 participants per group would provide 80% power to detect a significant difference in body weight between the TRE, CR, and control groups by month 6, using an overall F-test from a one-way ANOVA with α = 0.05, effect size of 0.4096, and a common standard deviation of 7%. We anticipated a dropout rate of 15%. Thus, we aimed to recruit 75 participants (n = 25 per group), assuming that 63 participants (n = 21 per group) would complete the trial.

Data will be shown as mean (95% CI) unless otherwise noted. A two-tailed P value of less than 0.05 will be considered statistically significant. We will conduct an intention-to-treat analysis, which will include data from all participants who underwent randomization. Results will be reported by intention-to-treat analysis unless indicated otherwise.

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13.0 Regulatory requirements

13.1 Informed consent: Consent will be obtained prior to the administration of the screening questionnaire by a member of the study team (Vicky Pavlou or key research personnel identified in Appendix P). The informed consent document will be stored in a locked filing cabinet at the Human Nutrition Research Center (1919 W Taylor St, Room 121C). All study team members have completed the CITI and HIPAA training courses.

13.2 Subject confidentiality: To protect subject identity, code numbers only will identify all laboratory specimens, evaluation forms, reports, and other records. Subject identities will not be used in any reports or publications resulting from this study. All patient records will be kept in locked files; code sheets linking the patient's name to a patient identification number will be stored separately in a locked file cabinet. Only study personnel will have access to the files.

13.3 Unanticipated problems: Reporting of adverse events will follow requirements mandated by the University of Illinois Office for Protection of Research Subjects (OPRS). The OPRS will receive a verbal or e-mail report of any serious adverse event (SAE) that occurs during the conduct of the study within 48 hours. This will be followed by a detailed written report within 10 working days. In addition, any incidents or problems involving the conduct of the study or patient participation, including problems with the recruitment and/or consent processes will be reported within 10 working days.

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2. Statistical analysis plan

For the sample size calculation, we estimated that TRE and CR groups would reduce body weight by 7% and 3% (extrapolated from our pilot data), respectively, by month 6, versus controls (no change in body weight). We calculated that n = 21 participants per group would provide 80% power to detect a significant difference in body weight between the TRE, CR, and control groups by month 6, using an overall F-test from a one-way ANOVA with α = 0.05, effect size of 0.4096, and a common standard deviation of 7%. We anticipated a dropout rate of 15%. Thus, we aimed to recruit 75 participants (n = 25 per group), assuming that 63 participants (n = 21 per group) would complete the trial.

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