Protocol Cover Page with Statistical Analysis Plan

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Protocol Title: The effects of time restricted feeding on AGE-RAGE signaling in women at high risk for breast cancer (TREC study: Time Restricted Eating on Cancer risk)

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PROTOCOL TITLE:

The effects of time restricted feeding on AGE-RAGE signaling in women at high risk for breast cancer (TREC study: Time Restricted Eating on Cancer risk)

PRINCIPAL INVESTIGATOR:

Harsha Karanchi

1.0 Objectives / Specific Aims

Pre-diabetes and post-menopausal state are associated with increased breast cancer risk (1, 2). Prolonged nighttime fasting duration may be associated with reduced breast cancer risk (3, 4). The underlying mechanistic aspects of prolonged overnight fasting duration and relationship to breast cancer risk is not yet known.

Advanced glycation end products (AGEs) are reactive metabolites that accumulate in tissues as we grow older. RAGE (receptor for AGE) is a cell surface protein that on AGE binding, leads to the transcriptional activation of inflammatory cascades and further RAGE upregulation. This cyclical pathway has been associated with the development of cancer (5, 6). Soluble RAGE isoforms (sRAGE) of the full-length RAGE are a heterogeneous pool of solubilized receptors which act as decoy receptors for AGE to attenuate RAGE signaling. sRAGE thus acts as a host defense mechanism with potential to reduce AGE-RAGE toxicity, is impaired by pre-diabetes(7), and shows an inverse correlation with cancer risk (8, 9).

Based upon our supporting data (10), time restricted feeding (TRF) may play a role as a therapeutic modality to decrease breast cancer risk by reducing AGE burden and enhancing AGE clearance mechanisms through increases in sRAGE levels: to reduce toxic AGE-RAGE axis effects.

The objective of this study is to assess the impact of TRF on AGE-RAGE toxicity in women at higher risk of breast cancer.

We hypothesize that time restricted feeding induced increases in soluble receptor for advanced glycation endproducts (sRAGE) may represent a cancer risk modification by reducing AGE-RAGE toxicity in post-menopausal women with pre-diabetes.

Specific Aim: To conduct a pilot Randomized Controlled Trial (RCT) designed to measure the effect of TRF on AGE-RAGE toxicity in postmenopausal women with pre-diabetes.

Pre-diabetes is a global problem, but is particularly prevalent in the United States, with 47.1 million adult women affected (31.2%). There is increasing evidence for pre-diabetes as an independent breast cancer risk factor, specifically in postmenopausal women. This aim will directly address the impact of TRF in this high-risk population, over a 3-month period and will evaluate plasma AGE and sRAGE levels, dietary AGE intake, and renal clearance of AGE.

In a proof of concept study, we will examine effect of TRF (n:22) vs control without TRF (n:22) in postmenopausal women with pre-diabetes (HbA1C 5.7-6.4% and/or fasting glucose 100-125 mg/dL). Plasma markers will be collected from each subject and stored for future analysis

Conducting the pilot study will enable us to obtain important feasibility and adherence information.

2.0 Background

Most energy restriction research in cancer has involved continuous energy restriction (11, 12). However, compliance to continuous energy restriction has been difficult to achieve. Alternatives include intermittent fasting (IF) regimens including time restricted feeding (TRF).

Time restricted feeding is an emerging and popular dietary pattern, provides a consistent daily pattern to feeding and fasting, and increases overnight fasting duration (13, 14). Studies show erratic eating window of > 15 hours per day in > 50% of people (15, 16), thus, a large proportion of individuals have a narrow overnight fasting window. Prior studies have shown that overweight or obese individuals with eating window of ≥ 14 hours can safely adopt an 8-10 hour interval of time restricted eating over several weeks and achieve metabolic benefits (16-18).

Prolonged nighttime fasting duration may be associated with reduced breast cancer risk (3, 4). The underlying mechanistic aspects of prolonged overnight fasting duration and relationship to breast cancer risk is not yet known.

NIH funded clinical trials are currently recruiting breast cancer patients to investigate the effects of IF regimens, with concomitant anticancer treatments (ClinicalTrials.gov Identifier: NCT03162289, NCT03595540), and immunologic and metabolic effects in the preoperative and post-operative setting (NCT03454282). A limitation of time restricted feeding studies is that they use controlled settings, include provision of meals, lack applicability to real life settings, and have not evaluated breast cancer risk (18-20).

We are exposed to high levels of advanced glycation end products (AGEs) through the lives we lead and the foods we eat. The pathogenic accumulation of AGEs on biological macromolecules is a hallmark of the aging process which leads to organ functional decline and chronic disease burden (21).

RAGE (receptor for AGE) is a cell surface protein that on AGE binding, leads to the transcriptional activation of inflammatory cascades and further RAGE upregulation. This cyclical pathway has been associated with the development of cancer (5, 6). Soluble RAGE isoforms (sRAGE) of the full-length RAGE are a heterogeneous pool of solubilized receptors which act as decoy receptors for AGE to attenuate RAGE signaling. sRAGE thus acts as a host defense mechanism with potential to reduce AGE-RAGE toxicity, is impaired by pre-diabetes (7), and shows an inverse correlation with cancer risk(8, 9).

In subjects with type 1 diabetes, sRAGE increases following a single overnight fast (22).
A high impact finding of our animal studies is that dietary-AGE induced increases in breast tumor growth are restricted by TRF, independent of total dietary AGE (10). Dietary-AGE mediated increases in breast tumor growth were dependent upon the stromal expression of the transmembrane receptor for AGE (RAGE). TRF lead to an increase in circulating RAGE, potentially indicating less membrane bound RAGE to mediate AGE toxicity.

Based upon our supporting data, TRF may play a role as a therapeutic modality to decrease breast cancer risk by reducing AGE burden and enhancing AGE clearance mechanisms through increases in sRAGE levels: to reduce toxic AGE-RAGE axis effects.

Pre-diabetes affects 31.2% of adult women in the U.S. (23). In postmenopausal women, metabolic syndrome is associated with 25% higher risk of incident breast cancer (2), and a two-fold higher breast cancer mortality (1). Hyperinsulinemia and insulin resistance, the hallmark of pre-diabetes, may be a better predictor of breast cancer risk compared with BMI (24). No previous study has investigated the role of TRF on AGE-RAGE associated breast cancer risk factors.

The results of this study are expected to directly impact studies of breast cancer risk modification by time restricted feeding.

Our pilot study will obtain important feasibility information on process measures, including retention rate, virtual visits with psychologist or dietician attended, adherence with food frequency questionnaires and overall adherence to eating pattern assigned (TRF vs control) and the effect of TRF on measures of glycemia measured using the Freestyle Libre Pro CGM.

Rationale for use of Freestyle Libre Pro Continuous Glucose Monitor (CGM): In post-menopausal women with pre-diabetes, we are studying the effect of time restricted eating on breast cancer risk biomarkers. One of our secondary outcomes is the effect of time restricted eating on measures of glycemia measured through CGM during baseline and end of study.
Rationale:
1. Metabolic syndrome which involves glucose dysregulation/hyperglycemia in pre-diabetes range is a marker for risk of breast cancer in post-menopausal women (1, 2).
2. Time restricted eating has been shown in studies of subjects with overweight and obesity to decrease fasting, mean and nocturnal glucose levels obtained through CGM. Other clinical trials have used Freestyle Libre Pro CGM in patients with metabolic syndrome (25), but not in the context of breast cancer risk biomarkers and not in a controlled trial, which our pilot study aims to do. Intervention studies in post-menopausal overweight women without diabetes but at high risk for breast cancer have used CGM to guide eating episodes and reported good compliance with CGM use and participants preference to wear CGM again (26).
3. Prolonged overnight fasting which our intervention: time restricted eating aims to achieve, has potential for reduction in breast cancer risk (4), but mechanisms remain uncertain.
One of the mechanisms may be improved glycemia from prolonged nighttime fasting (3). This requires study in randomized trials.
To study potential underlying mechanisms: In addition to advanced glycation end products as primary outcome, we aim to study glycemia as secondary outcome. Glycemia is best measured through continuous glucose monitoring rather than fasting glucose measured at Visit 1 and Visit 2, or self-monitoring of finger stick glucose by glucometer. The Freestyle Libre Pro CGM has been validated for 14 days of continuous use with factory calibration, will continuously record glucose for 2 weeks during baseline and end of study. Participants will be blinded to CGM data. CGMs estimate glucose with high accuracy and correlates with venous or capillary blood glucose. Use of CGM will avoid self-monitoring of glucose by finger stick 4-7 times per day which has been used by previous studies but is burdensome.

Feasibility and adherence data will enable better future study designs and provide insight on ways to improve compliance. CGM data obtained will enable future larger randomized trials evaluating association of glycemia in pre-diabetes range with breast cancer risk and interventions to reduce this risk.

3.0 Intervention to be studied

Intervention: Timing of eating intervention, Group 1, TRF (daily eating period of 8 hours, before 8 PM); Group 2, Control without TRF (daily eating period ≥ 12 hours).

Duration of study: 14 day run in period, 3 month study duration.

Time restricted feeding is well tolerated with minimal side effects, from previous studies (17, 20, 27) and PI clinical experience. We will use a fasting period of 16 hours in the intervention group. In a previous controlled study, daily fasting for 18 hours was reported as well tolerated and not difficult, but eating all meals within 6 hour period was reported as more difficult than the challenge of fasting for 18 hours per day. Participants reported eating within an 8 hour daily period would be feasible for most people (20). Based on these feasibility and acceptability results, we will use an eating period of 8 hours.

Overall, the risks involved in this study are truly minimal and therefore based on the potential personal benefit to the enrolled volunteers and the overall knowledge gained, reasonable.

Protection against side effects.
In a previous early time restricted feeding study, lower desire to eat, lower capacity to eat, and increased fullness was reported in the evening, with no differences in the morning, and no increase in hunger at any time, in intervention group compared with control (20).

Subjects in our study will be given the following instructions: “If you get sensation of hunger or light headedness in the fasting period, this is most likely just dehydration. Drink noncaloric fluids and see if the sensation goes away. In most cases, this sensation only lasts few minutes when you hydrate yourself. Your body is getting used to the new pattern of eating and these episodes will get better with time, usually within 7 days. With the new eating pattern, your hunger will be reset to when your body is ready to eat or the new eating period, rather than during times of false signals from prior erratic eating patterns.”

Subjects in the intervention group may take time to adapt to new time restricted feeding pattern, this individualized adaptation will be allowed for in our study, and subjects will be counseled on the same. Subjects in both groups will be supported by virtual visits with psychologist or dietician, through established doxy.me platform or phone calls, at defined intervals throughout the study: pre-intervention, weekly for first month, alternate weeks for second month and once during last month of study. Subjects will receive weekly phone calls from research coordinator as needed. Such ongoing contact with psychologist or dietician and research coordinator will help address any discomforts and enable use of behavioral strategies to address.

4.0 Study Endpoints

Primary End Point: Fasting plasma AGE, sRAGE levels;
Assess feasibility and adherence to time period of eating recommendation in both study groups.
Secondary End Points:
Fasting IGF-1 levels;

Fasting insulin levels;
Glasgow Prognostic Scoring System: cumulative inflammation-based cancer-prognostic marker (CRP and albumin concentration);
24 hour urinary AGE levels;
CGM derived metrics: mean glucose, standard deviation (SD) of mean glucose values, co-efficient of variation (CV%) of sensor glucose levels, glucose management indicator (GMI%) from CGM data;
Adherence to virtual visit with psychologist or dietician;
Adherence to time period of eating recommendation in both study groups: self-report during virtual visits and through food photography/annotated entries;
TRF affected sleep patterns as assessed using American Academy of Sleep Medicine 2-week sleep diaries.

5.0 Inclusion and Exclusion Criteria/ Study Population

The pilot RCT is a proof of concept study, in postmenopausal women (no menstrual periods in the preceding 12 or more months) with pre-diabetes (A1C 5.7-6.4% and/or fasting glucose 100-125 mg/dL).

Intervention: Timing of eating intervention, Group 1, TRF (daily eating period of 8 hours, before 8 PM); Group 2, Control without TRF (daily eating period ≥ 12 hours).
Duration of study: 14 day run in period, 3 month study duration.

Patient Selection: Patients must meet all of the following criteria to be eligible for enrollment.

Inclusion Criteria:
Age ≥ 40 and ≤ 67;
Postmenopausal women (no menstrual periods in the preceding 12 or more months) with pre-diabetes (A1C 5.7-6.4% and/or fasting glucose 100-125 mg/dL). A1c lab and/or fasting glucose criteria will need to be met within 12 months of signing consent form. Lab results can be obtained from prior lab result or study prescreening testing;
Own a smart phone with internet connection and capable of receiving and sending text messages and taking photographs:

Exclusion Criteria:
Tobacco use (current or within last 2 years);
Active malignancy or history of cancer;
History of known liver disease (by serology: AST or ALT ≥ 3 times above upper limit of normal determined by lab review, imaging or biopsy: determined by patient history);
History of kidney disease (patient history and/or eGFR less than 45 mL/min/1.73m²)
History of diabetes mellitus;
History of cardiovascular disease (MI, CHF);
Current prescription medication use for diabetes;
Medication affecting glucose metabolism or appetite or immunosuppression;
Dietary restrictions: currently following vegetarian or vegan dietary pattern;
Currently following intermittent fasting or time restricted feeding pattern or use in the last 3 months;
Night shift worker (work schedule does not involve any period of work from 10 PM to 5 AM either on a regular or rotating basis);

History of weight loss >5% in the last 3 months;
History of weight loss surgery;
BMI≥40 kg/m² exclusion;
After informed consent and run in period: Insufficient documented food photography/annotated entries (does not log at least two entries a day for 10 of 14 days) during run in period will be excluded from randomization into the intervention period.

Exclusion of Men: the research question addressed is relevant to only one gender

Exclusion of Children: the condition is rare in children as compared to adults

Inclusion of Individuals Across Lifespan, rationale for age group selection:
The proposed clinical trial is specific for postmenopausal women as the group of women at high risk for breast cancer and as we are focused on breast cancer prevention, we have excluded Age <40 or >67. Minimum age of 40 was chosen to exclude women with premature menopause (primary ovarian insufficiency) who are different in hormonal milieu compared to women with menopause around usual age of menopause. Maximum age of 67 was chosen for the following reasons:
1. Safety of time restricted feeding in age group above 70 is not known, will need a separate study.
2. Risk of breast cancer rises acutely above age 70 (28) and implementing preventive measures in this age group might not have desired impact.
3. Given the primary outcome of the study is assessment of AGE-RAGE axis, and AGE-RAGE axis abnormalities increase with age and glucose dysregulation , a wide age range at inclusion increases heterogeneity of the baseline plasma AGE and sRAGE levels with potential for wide confidence interval and decreased power to detect differences with intervention, this will likely not be resolved by randomization given the pilot nature of our study. Our future trials will however work to address this limitation, having gained insight from the pilot study.
4. Mean age of menopause is 51.4 years and increasing upper limit of inclusion to 67 will make study accessible to more women who are otherwise eligible and have expressed interest in our study, will also help increase the pool of eligible participants for study and help with increasing study recruitment.

Dr. Karanchi, Dr. Cornier, Dr. Lal, Dr. O'Neil have long standing experience in working with this age group, and this includes clinical and research experience.

MUSC SCTR (South Carolina Clinical and Translational Research Institute) Nexus clinic provides facilities that are appropriate for all age groups.

Given the focus of study is on role of time restricted feeding in breast cancer prevention, average age of menopause is age 51 with considerable variability, and risk of breast cancer increases with postmenopausal state and older age, we feel that our inclusion of women aged between 40 to 67 best captures the cohort where prevention lifestyle measures would be most impactful.

6.0 Number of Subjects

We plan to enroll up to 60 participants with the goal that 44 participants will meet criteria for randomization following the run in period. Once 44 participants have met criteria for randomization we will halt enrollment.

7.0 Setting

Single center study: MUSC

SCTR Nexus clinic at MUSC will be used for study visits, vital signs, blood and urine collection. Blood collection and prescreening testing may be done at MUSC facilities or other prescreen locations.

8.0 Recruitment Methods

We will use TriNetX, a healthcare research network tool through South Carolina Clinical and Translational Research Institute (SCTR), and MUSC EPIC recruitment reports to identify potential subjects.. We ran a query with our inclusion and exclusion criteria for patients receiving care at MUSC and identified 2800 potential subjects.

With IRB approval, a Research Data Request will be submitted to obtain a recruitment report of all MUSC patients who potentially meet eligibility criteria. Chart review will be conducted for research purposes. The MUSC cold contact policy will be used for recruitment. The study staff will not contact any potential participants that have opt-ed out of receiving contact about research. The study staff may contact potential participants by telephone using an IRB approved script, MyChart message, SMS text, e-mail or letter. If a potentially eligible patient has a clinic appointment, the study team may notify the patient's provider and the provider may ask the patient if they are interested in speaking with the study team. Study team may email MUSC providers informing them of study, use of electronic medical record (EMR) alerts and attach IRB approved study patient recruitment materials. For MUSC providers who agree, they will receive EMR alerts for potentially eligible patients. If a potentially eligible patient gives verbal permission to the provider, their information may be provided to the study team for contact about the study.

Advertising will be used in the form of posters and brochures. Posters may be posted on MUSC Facebook pages. Study information may be placed on MUSC digital screens. Recruitment may be done through community outreach events. MUSC providers may also give patients cards with study information and study team contact. When a potential participant contacts the study team, their MUSC medical record will be reviewed for eligibility.

Numbers of potential participants and reasons for non-participation will be tracked throughout the recruitment process to identify areas for improvement. We will evaluate recruitment strategies with potential participants and seek feedback on barriers/reason for non-participation in clinical trial. Participants can choose to not answer this question. Screening logs will track reasons for ineligibility or non-participation, which will help identify barriers to trial success overall as well as barriers that limit enrollment of particular populations (e.g. based on age, race/ethnicity, zip code). Recruitment and adherence will be tracked by patient demographics.

9.0 Consent Process

Informed consent will be obtained in-person, remotely using MUSC's Teleconsent platform or by telephone. Only subjects who are able to consent for themselves will be asked to participate. The HIPPA authorization is part of the informed consent document.

If the informed consent is obtained in-person, the consent discussion will take place in a private area and the potential participant will be given the opportunity to read the consent and ask questions. The participant will be given a signed copy of the consent form.

If the consent is obtained using MUSC's Teleconsent platform using doxy.me, the potential participant will receive a web link which allows study personnel to present the consent form and allows the potential participant to ask questions and read the consent form. The consent form is signed by both parties using this platform and the participant may print a copy for their record.

If the consent is obtained via telephone, the consent form will be sent to the potential participant. Study personnel will speak with the potential participant via a telephone call to discuss the consent form and answer any questions. The participant will then sign the consent form and send the consent form to study personnel. Study personnel will sign the consent form at the time of the phone discussion and attach the participant's signed consent form when received to have a fully executed consent document.

There is no waiting period between informing the prospective subject and obtaining the consent, however, the informed consent may be mailed or e-mailed to a potential research subject for review and the consent process completed at another time . If the subject no longer wishes to participate, they may contact the study via the address on the HIPAA authorization section to withdraw their participation. All signed consent forms will be kept in a locked office.

A verbal prescreening consent may be obtained for a fingerstick hemoglobin A1C. The participant will be provided a prescreening information sheet.

10.0 Study Design / Methods

Study Design: Pilot, intervention, proof of concept, RCT, two arm, parallel design, single center study.

Timing of eating intervention

Potential study subjects will be screened with phone interview.

All efforts will be made to abstract laboratory measurements (HbA1C, fasting glucose) from previous standard of care testing, to evaluate for study eligibility. If a patient is otherwise eligible but does not have a HbA1C or fasting glucose result in the last 12 months, the study team will screen such participants with point of care hemoglobin A1C testing, after obtaining verbal consent for such screening. Participants that qualify will not be obliged to participate in clinical trial unless they provide informed consent for the clinical trial. Participants will be provided a handout on meaning of test results and guided to follow up with primary care provider.

After informed consent of individuals meeting inclusion and exclusion criteria, participants will undergo 14 day run in period, and then randomized to intervention or control study arm.

Intervention group: Group 1, TRF (daily eating period of 8 hours, before 8 PM);
Control: Group 2, Control without TRF (daily eating period ≥ 12 hours).

Run in period: 2 weeks
During run in period: Participants will receive automated, daily text messages from Twilio through REDCap with the request to upload photos and/or descriptions of each meal or snack. Date/time data will be programmatically extracted from the photos and inserted into participant upload records to ensure the photo was taken at the appropriate time

Randomization: at end of run in period. Up to 44 participants that meet inclusion criteria following the run- in period will be randomized. Dr. Hunt will use a random number generator to create a blocked randomized list. Dr. Hunt will assign a random ID and use this to label envelopes which will contain information on what arm of the study the participant will be randomized to. Group allocation will be indicated within the envelope. Participants will be notified of randomized group by phone.

Duration of study: 12 weeks (+/- 7 days)

8-hour time restricted eating in the intervention arm limits daily dietary intake to consistent self-selected 8-hour window, creating a 16 hour nightly fast.

During both run in and intervention period, participants will not be instructed to change diet quality, quantity or caloric content, or physical activity.

Visit 1, Week 0: Vital signs, height, weight, waist circumference, blood samples, 24 hour urine collection, questionnaires.

A Freestyle Libre Pro continuous glucose sensor (CGM) will be inserted on the participant's upper arm to measure interstitial glucose. This sensor will record glucose every 15 minutes but will not display the value to the participant. The participant will be instructed to remove the sensor after 14 days and return the sensor to the site in a provided zip-top bag and padded self -addressed stamped envelope. The participant will be given instructions about wearing and removing the sensor.

The participant will be trained on responding to automated, daily text messages from Twilio through REDCap with the request to upload photos and/or descriptions of each meal or snack.

Participants will be responsible for all text message/data rates charged by their phone provider.

Week 0-2: 14 day run in period will serve as baseline before randomization.
Run in period will be used to determine whether patient is able to sufficiently log dietary intake via text messages. Participants will be discontinued from the study after run in period, if there is insufficient

logging of dietary intake (does not log at least two entries a day for 10 of 14 days) during run in period and will not be randomized into the intervention period.

At the end of baseline run in period: subjects will be randomized to intervention or control study arm. Intervention arm: Subjects will be instructed to self-select daily 8 hour eating window ending before 8 PM each day. Subjects in the intervention arm will be allowed to eat outside the 8-hour window up to 2 days/week, to allow social commitments deemed necessary by subjects.

Control arm: Subjects will be asked to continue current daily eating period ≥ 12 hours.

Participants may consume caffeine (without additional nutritional content such as cream, sugar, or artificial sweeteners) outside the eating window as needed, and document via text message.

Participants will continue to receive automated, daily text messages from Twilio through REDCap with the request to upload photos and/or descriptions of each meal, snack and beverage consumed.

Week 12: A CGM sensor kit and instructions will be mailed to participant. A virtual visit or phone call will be done by trained study staff to help place CGM sensor on the arm two weeks prior to visit 2.

Reason for video visit for freestyle libre insertion by patients during week 12 of study: We aim to limit participant burden and also limit costs of additional visit.
Participant will be trained during Visit 1 when first CGM is inserted, Video/phone visit for second CGM insertion will re-educate participants and help support insertion by participants.

The Freestyle Libre was designed to be inserted by medical providers or patients and has an easy use to applicator. We have experience with patients inserting the sensor. During the pandemic, other studies have used virtual visits to assist participants with CGM sensor insertion (29).

Visit 2, Week 14 (+/- 7days) Vital signs, weight, waist circumference, blood samples, 24 hour urine collection, questionnaires. The Freestyle Libre Pro CGM inserted by participants during week 12 will be removed during Visit 2.

Visits with psychologist or dietician: through phone call or established doxy.me platform: pre-intervention, weekly for first month, alternate weeks for second month and once during last month of study, and at end of intervention.
Week 3 after randomization before start of intervention.
Week 4
Week 5
Week 6
Week 8
Week 10
Week 12
Week 14 before end of intervention.

The study coordinator may make phone calls and send text messages as needed to subjects to encourage compliance.

Visit 1 and Visit 2: Food Frequency Questionnaires (FFQ), sleep and exercise questionnaire.

Schedule of Events Table:

	Phone call	In-person or remote contact	Visit 1, Enrollment	End of Run in	Weeks 3,4,5,6,8, 10,12, 14	Visit 2 Week 14 (+/- 7 days)
Prescreening verbal consent, if indicated		X				
Inclusion/ Exclusion	X					
Informed Consent		X				
Dispense urine collection supplies		X	X			
Fasting blood draw			X			X
24 hour urine collection			X			X
Text message all dietary intake			X	X	X	X
Placement of CGM sensor			X		Week 12 only	
Removal of CGM sensor				X		X
Vital signs			X			X
Questionnaires			X			X
Randomization				X		
Telemedicine or phone visit with psychologist or dietician				X	X	
Phone call by study coordinator to			As needed	throughout	the	study

encourage compliance						

Protocol:

Prescreening point of care testing will be done at MUSC facilities or recruitment outreach events. Point of care testing will be done by the study team.

SCTR Nexus clinic at MUSC will be used for study visits 1 and 2, to obtain vital signs, blood and urine collection. Subjects will be asked to avoid consumption of food, drink or caffeine, except water, for 12 hours prior to lab collection and avoid alcohol and exercise for 2 days before lab collection. Fasting blood samples (drawn after 12 hours of fasting in both groups to help match fasting duration), 24 hour urine collection, and food frequency (FFQ), power of food, food cravings, hunger and satiety, sleep and exercise questionnaires will be obtained before start of and at end of intervention. Subjects will be instructed to bring completed 24 hour urine collection to Visit 1 and Visit 2.

Patients will be provided remuneration for participation for each of the two visits and given food voucher after fasting lab draws.

Subjects in both groups will also fill out 2 week sleep diaries during 2 week run in period and last 2 weeks of intervention.

Subjects in both groups will have virtual visits with our psychologist or dietician, through phone call or established doxy.me platform, at defined intervals throughout the study: pre-intervention, weekly for first month, alternate weeks for second month and once during last month of study. Our psychologist or dietician will use data on patient self-report to enhance compliance with study group recommendations by use of motivational interviewing strategies. The study coordinator will make phone calls to subjects as needed to encourage compliance.

Blood and urine analysis: Plasma AGE and sRAGE (includes the cRAGE and esRAGE isoforms) will be measured by ELISA (as described in SA2). The Glasgow Prognostic Scoring System is a cumulative inflammation-based cancer-prognostic marker consisting of an assessment of serum elevation of CRP and decrease in albumin concentration by ELISA.

Samples for comprehensive metabolic panel, IGF-1, lipid panel, fasting insulin, uric acid, hemoglobin A1C, C-reactive protein and Apolipoprotein B will be obtained at baseline and at end of study (visit 1 and visit 2).
24-hour urinary AGE will be measured by fluorescence assay.

Urine creatinine will be assessed.

To assess renal handling and clearance, eGFR will be used to calculate AGE fractional excretion, and renal clearance of AGE will be calculated.

Dietary AGE analysis: Daily dietary AGE content from food frequency questionnaires will be estimated using methods we have published previously (30).

11.0 Specimen Collection and Banking

Fasting blood samples (drawn after 12 hours of fasting in both groups to help match fasting duration), 24 hour urine collection, will be obtained before start of and at end of intervention.

All samples will be coded, samples will have results associated with coded label.

The samples will be stored in Hollings Cancer Center Translational Science Laboratory.
Associated data will be stored on Division of Endocrinology, Diabetes and Metabolic Diseases MUSC drive and the Department of Public Health Sciences MUSC Server.

The specimens will be stored for a minimum of 2 years (the duration of the study) and or/until the samples are depleted on use for analysis.

Dr. Karanchi will have access to the specimens and associated data including data from analysis.

The research coordinator will transport research specimens from SCTR nexus clinic, using a sealed container containing biohazard label. The samples will be carried in person to the Hollings Cancer Center Translational Science Laboratory for processing and storage in -80° C freezer.

All specimens are for use by Dr. Karanchi, his direct collaborators at MUSC and collaborators at Virginia Commonwealth University. Only Dr. Karanchi will have the authority to release samples for analysis. All samples will be coded. We will use a Materials Transfer Agreement to send samples to collaborators at Virginia Commonwealth University. The samples sent to Virginia Commonwealth University will be de-identified.

No samples will be used for genetic analysis. No samples will be saved from the prescreening point of care testing.

12.0 Data Management

Statistical Considerations: Sample Size Determination: The sample size justification focuses on the primary aim of obtaining effect size and variance estimates required to plan an RCT. Effect sizes will be estimated via 95% confidence intervals within and between groups (primary analyses). For example, for our primary outcome of AGE level, we will estimate mean levels within the intervention and usual care arms of the study. For all continuous outcome variables, within each study arm, after accounting for a 10.0% attrition rate post randomization n1=n2=204, we anticipate that *within group* mean levels can be

estimated with precision ±0.47s (s=standard deviation within each group). For *between group* comparisons, precision of effect size estimates is ±0.64s**p** comparing the intervention to usual care arm (s**p** is the pooled standard deviation across groups being compared).

Analysis: Statistical analysis for the pilot trial relate largely to our aim to obtain preliminary feasibility findings. We focus on our primary intervention outcome measures in this analysis section which are AGE and sRAGE levels, 3 months after study entry. Effect size estimates of differences in AGE and sRAGE levels will be obtained using 95% confidence interval estimates of differences between means. If the difference in the magnitude of the mean in the exposure categories is sufficiently large to suggest a clinically meaningful effect, the difference along with its standard deviation will provide the necessary effect size measure for accurately determining sample size in the subsequent RCT. Similarly, the magnitude in the difference in changes (along with upper and lower 95% CI limits)
can be used to indicate if the intervention produced a detectable clinically meaningful effect over our 3-month follow-up time.

Feasibility and adherence to time period of eating recommendation in both study groups will be measured by percentage of days logged that participants ate within their eating window.

Exploratory Analyses: The objective of these exploratory analyses is to determine trends toward statistical significance rather than confirm/refute hypotheses with adequate statistical certainty (power). Descriptive statistics and frequency distributions will be calculated as appropriate for all demographic and baseline clinical variables for the total sample and by intervention and control groups. Data will be transformed, or semi-parametric or nonparametric analysis procedures will be used as needed. Baseline values for variables will be compared for imbalance between intervention and control group using one-way analysis of variance (or nonparametric Kruskal-Wallis one-way ANOVA) followed by a pairwise multiple comparison procedure (e.g. Tukey's procedure), if indicated.
Categorical baseline variables will be compared using chi-square analyses.

The primary analyses will use the intent-to-treat (ITT) sample, comprised of all randomized participants. The completer sample will comprise subjects who complete the 3-month follow-up. Analyses will be carried out separately for the ITT sample and for the completer sample. Missing data for this study arising from premature exits (dropouts) will result if a patient’s participation is terminated before the 3 months study period is completed. Participants will not be discontinued from the study because of non-adherence; all will remain in the study unless consent is withdrawn or there are concerns regarding patient safety. We will adopt several approaches to dealing with missing data for the ITT analysis set: for continuous outcomes requiring a single endpoint end-of-study value (change from baseline to 3 months), we will use multiple imputation methods to impute missing 3-month values. We will evaluate the sensitivity of study conclusions to the presence of missing data by comparing the results of ITT to the completer analyses and the imputation results. We will use information on premature exits/missed appointments, food photography and dietary logs as indicators of participation, feasibility and fidelity of the treatment modalities being compared. Because this is a feasibility study, no adjustment will be made for analyses using the primary or secondary outcome measures.

Data Security:
Data confidentiality, Data Acquisition and Management

We plan to use REDCap for data capture and management. REDCap (Research Electronic Data Capture) is a software toolset and workflow methodology for electronic collection and management of research and clinical trial data. REDCap provides secure, web-based flexible applications, including real time validation rules with automated data type and range checks at the time of entry. Exports are made available for several statistical packages including SPSS, SAS, SATA, R and Microsoft Excel.

The study recruitment report, will be housed separately from the data capture in REDCap. Study staff will not have access to the recruitment report after recruitment is complete.

Surveys:
The system allows the research team to create and design online surveys and engage respondents using a variety of notification methods.

Security, Privacy, and Confidentiality
The underlying REDCap database is hosted in a secure data center at MUSC, a secure environment for data systems and servers on campus, and includes redundancy, failover capability, backups and extensive security checks. The system has several layers of protection including, user/group account management, "Data Access Groups" which allow data to be entered by multiple groups in one database with segmented user rights for entered data, audit trails for all changes, queries and reports, and Secure Sockets Layer (SSL) encryption.

The data center in which the REDcap servers are housed has strict access control; only authorized core personnel may access the facility un escorted. Only authorized users are allowed to connect to the network, and the security of the network is actively monitored. Power and environmental controls have several layers of backups, from interruptible power supplies to alternate and redundant feeds to the local utility company. The REDcap system administrator contributes to the maintenance of institutional disaster recovery and business continuity plans. Load balancers and a highly fault tolerant SAN infrastructure contribute to high availability. All transactions are securely delivered to the application using SSL (SHA-1 with RSA Encryption; 2048-bits). It is then transmitted internally (behind the firewall) to the database server. All transactions are logged at the server layer (http logging), application layer (REDCap logs activity to a database table), and the database layer (using both query and binary logging).

The REDcap system relies upon the institution's identity and access management infrastructure. Password complexity, history and expiration standards are implemented at the institutional level. Access to the data is managed by institutionally sponsored login IDs. All personnel must pass an employment background check before being issued an ID. Access to individual REDCap projects (and their data) is managed by the owner of the project.

The physical security of the data center is actively monitored 24x7 by security personnel using closed—circuit video. The institution actively logs and monitors all communication to the application server (multiple firewall layers prevent direct external communication to the database server) and the system owner is alerted to any unusual activity. If warranted, the institution will immediately as well as automatically ban offending IP addresses at the perimeter before they reach the application server. The application itself also rejects and bans IP addresses of anything it considers abnormal access.

Twilio (https://www.twilio.com/) is an IS-Security approved text message platform for research at MUSC and is integrated with REDCap. No data are stored when using Twilio; it only acts as a pass-thru. Personal health information is not sent via text message; only the phone number is used to send the message including a unique link to their web accessible food survey submission.

13.0 Provisions to Monitor the Data to Ensure the Safety of Subjects

DATA AND SAFETY MONITORING PLAN
MUSC has a federal wide assurance for research with human subjects, and is in compliance with federal policy governing use of human subjects. Individuals involved in human subject research at MUSC are required to complete the Collaborative IRB Training Initiative offered online by the University of Miami. All human subject protocols are reviewed through an academic Institutional Review Board (IRB) process that has been accredited by the Association for Accreditation of Human Research Protection Programs. MUSC has an integrated University Compliance Program that is organized via an active compliance liaison and committee structure for financial, clinical, academic and research compliance. This involves universal employee and professional training as well as a confidential Compliance Helpline to encourage all members of the MUSC community to ask questions or voice concerns about topics such as coding and billing, research integrity, professional ethics, human subjects research, animal care and use, biological safety, potential conflicts of interests, patient confidentiality and laws and regulations. Institutional research compliances include a training and certification process for all key personnel engaged in research involving human subjects, vertebrate animals or biohazardous substances, as well as integration into curricula of appropriate instruction in the Responsible Conduct of Research.

Informed consent will be obtained in-person or remotely using MUSC's Teleconsent platform or by telephone. Consent will only be obtained from the participant. Children and cognitively impaired adults will not be enrolled.

If the informed consent is obtained in-person, the consent discussion will take place in a private area and the potential participant will be given the opportunity to read the consent form and ask questions. The participant will be given a signed copy of the consent form.

If the consent is obtained using MUSC's Teleconsent platform using doxy.me, the potential participant will receive a web link which allows study personnel to present the consent form and allows the potential participant to ask questions and read the consent form. The consent form is signed by both parties using this platform and the participant may print a copy for their record.

If the consent is obtained via telephone, the consent form will be sent to the potential participant. Study personnel will speak with the potential participant via a telephone call to discuss the consent form and answer any questions. The participant may then sign the consent form and send the consent form to study personnel. Study personnel will sign the consent form. A signed copy of the consent form will be provided to the participant..

Example 1:
a. Our data and safety and monitoring plan will monitor all patients to ensure the safety of participants and the validity and integrity of the data. Protection to participants will be in accordance with guidelines set forth by NIH. Dr. Cornier has vast experience in clinical trials and has completed several trials. The level of monitoring will be as rigorous as in the prior published clinical trials. All serious, unexpected, AND related adverse events will be reported to the IRB.
b. Study will be registered in ClinicalTrials.gov before the first subject is enrolled.
Any change in endpoints or analysis plan that occurs will be amended in ClinicalTrials.gov.
The reporting of summary results information (including adverse events) will occur no later than 1 year after the completion date.

Example 2:
All adverse events (AE) will be reported and monitored. The AE's will be characterized by: intensity/severity; expectedness; relatedness; frequency; outcome; treatment or action taken. Monitoring will be performed at each study visit by the study coordinator and PI, adverse events will also be monitored during regularly scheduled visits (pre-intervention, weekly for first month, alternate weeks for second month and once during last month of study) with the psychologist or dietician and such events will be reported to PI. All AEs will be recorded on an event form with physician/nurses/coordinator notes attached.
These will be reviewed by Dr. Karanchi and Dr. Cornier.
We will have local study team meetings on a quarterly basis to address the following areas: 1. Review progress of the research study, including participant recruitment, retention and adherence. 2. Review adverse events to determine whether there is any change to anticipated benefit-to-risk ratio of study participation, and whether the study should be continue as originally designed or be changed or terminated. 3. Review data forms, data quality and data confidentiality. Drs. Karanchi and Cornier will arrange for the meetings quarterly, make all materials available for review by the study team and be ultimately responsible for the meetings.

All serious, unexpected, AND related adverse events will be reported to the MUSC IRB within 10 working days of learning of the event.

Overall, the risks involved in this study are truly minimal and therefore based on the potential personal benefit to the enrolled volunteers and the overall knowledge gained, reasonable.

14.0 Withdrawal of Subjects

Participants will be discontinued from the study after informed consent and run in period, if there is insufficient text message logging(does not log at least two entries a day for 10 of 14 days) during run in period and will not be randomized in to the intervention period.

Participants will not be discontinued from the study because of non-adherence after randomization; all will remain in the study unless consent is withdrawn or there are concerns regarding patient safety. All such events will be reviewed by PI in coordination with study team.

The primary analyses will use the intent-to-treat (ITT) sample, comprised of all randomized participants. The completer sample will comprise subjects who complete the 3-month follow-up. Analyses will be carried out separately for the ITT sample and for the completer sample. Missing data for this study arising from premature exits (dropouts) will result if a patient's participation is terminated before the 3 months study period is completed.

Subjects may withdraw their consent by contacting the study team via the telephone number on the consent form.

15.0 Risks to Subjects

Potential Risks.

Loss of confidentiality: Protection of patient confidentiality is essential in human subjects research. A HIPAA compliant de-identification process will be utilized which includes a unique computer-generated study ID for each enrolled patient. Study binders will be maintained in locked physical facilities and only accessible to authorized study team members to protect patient privacy. No data are stored when using Twilio; it only acts as a pass-thru. PHI is also not sent via text message; only the phone number is used to send the message including a unique link to their web accessible food survey submission.

Risks associated with blood collection include momentary discomfort and/or bruising, infection, excess bleeding, clotting, or fainting is possible, although unlikely.

CGM sensor risk may include mild pain associated with sensor insertion, infection, redness, bleeding and reaction to adhesive such as swelling, rash, itching.

Risks and or discomforts of time restricted feeding may include increased sensation of hunger, headache, nausea, dizziness, fatigue. These risks and or discomforts are transient, can be related to dehydration and can be minimized by avoiding dehydration. There may also be an adaption period to minimize these risks. Time restricted feeding is well tolerated with minimal side effects (17, 20, 31), from previous studies and PI (Karanchi) clinical experience.

In our study, subjects in both groups will have virtual visits with our psychologist or dietician, through phone call or established doxy.me platform, at defined intervals throughout the study: pre-intervention, weekly for first month, alternate weeks for second month and once during last month of study. Our psychologist or dietician will use data on patient self-report to enhance compliance with study group recommendations and address any patient concerns by use of motivational interviewing strategies. The study coordinator will make phone calls to subjects to encourage compliance. Such ongoing contact with psychologist or dietician and research coordinator will help address any discomforts and enable use of behavioral strategies to address.

To allow for individual subject schedule and personal preference, we will allow patients in intervention group to self-select eating window of 8 hours to be completed by 8 PM.

We will use a fasting period of 16 hours in the intervention group. In a previous controlled study (20), daily fasting for 18 hours was reported as well tolerated and not difficult, but eating all meals within 6 hour period was reported as more difficult than the challenge of fasting for 18 hours per day. Participants reported eating within an 8 hour daily period would be feasible for most people. Based on these feasibility and acceptability results, we will use an eating period of 8 hours.

Overall, the risks involved in this study are truly minimal and therefore based on the potential personal benefit to the enrolled volunteers and the overall knowledge gained, reasonable.

16.0 Potential Benefits to Subjects or Others

Potential benefits to the study subjects.
Time restricted feeding has been previously shown to improve insulin resistance and inflammation. Interrupting AGE-RAGE signaling by TRF has potential for anti-inflammatory effect. Improvement in insulin resistance may lead to reduction in hemoglobin A1C and thus improvement in pre-diabetes. TRF has not previously been studied in relationship to AGE or sRAGE levels in the context of an experimental study of humans. Although our feasibility study is not powered to detect a statistically significant difference, we expect that the intervention group will demonstrate a clinically significant reduction in AGE and increase in sRAGE compared to the control group. Increases in sRAGE may represent a cancer risk modification by reducing AGE-RAGE toxicity, in this high-risk population.

It is possible that participating subjects in both groups will benefit from the regularly scheduled follow-up encounters with our psychologist or dietician. This may afford them an opportunity to express concerns regarding their health and wellness, and may result in subsequent visits with appropriate health care providers.

Overall, the risks involved in this study are truly minimal and therefore based on the potential personal benefit to the enrolled volunteers and the overall knowledge gained, reasonable.

Potential benefits to science and society.
The results of this study are expected to directly impact studies of breast cancer risk modification by time restricted feeding. Our pilot study will obtain important feasibility information on process measures, including retention rate, virtual visits with psychologist or dietician attended, adherence with food frequency questionnaires and overall adherence to eating pattern assigned (TRF vs control).
This feasibility and adherence data will enable better future study designs and provide insight on ways to improve compliance.

Time restricted feeding has the potential to be a practical population wide cancer prevention strategy. Research findings will have limited or unknown external validity unless we answer complex implementation and dissemination questions. Telehealth has great potential during attempts to implement many new research findings. All the above has potential to mitigate cancer disparities through a practical, widely applicable and cost effective novel strategy.

Importance of the knowledge to be gained.
Results from our studies will identify the role of TRF in interrupting AGE-RAGE signaling, provide insight into adherence to TRF intervention: in women at high risk of breast cancer.
Results will provide the basis for larger intervention studies, investigating sRAGE as a novel modifiable cancer risk factor by TRF. The overarching broad long-term objective is to find a practical population wide cancer prevention strategy: Target AGE toxicity through TRF; to reduce breast cancer risk, incidence and recurrence.

17.0 Sharing of Results with Subjects

Prescreening results will be shared with subject at the time of prescreening. Individual subject lab results are shared upon subject's completion of study. Study results will be shared with subjects at the conclusion of study.

18.0 Drugs or Devices (if applicable)

NA

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