Received: 23 May 2022 | Revised: 11 July 2022 | Accepted: 20 July 2022

DOI: 10.1002/oby.23579

ORIGINAL ARTICLE

Clinical Trials and Investigations

Obesity A Research Journal | THE OBESITY SOCIETY | WILEY

The basis and design for time-restricted eating compared with daily calorie restriction for weight loss and colorectal cancer risk reduction trial (TRE-CRC trial)

Kelsey Gabel[1,2] | Marian L. Fitzgibbon[2,3] | Cemal Yazici[2,4] | Peter Gann[2,5] | Maria Sverdlov[2,6] | Grace Guzman[2,5] | Zhengjia Chen[2] | Andrew McLeod[2,3] | Alyshia Hamm[1,2] | Krista A. Varady[1,2] | Lisa Tussing-Humphreys[1,2]

[1]Department of Kinesiology and Nutrition, University of Illinois Chicago, Chicago, Illinois, USA

[2]University of Illinois Cancer Center, Chicago, Illinois, USA

[3]Institute for Health Research and Policy, Chicago, Illinois, USA

[4]Department of Medicine, University of Illinois Chicago, Chicago, Illinois, USA

[5]Department of Pathology, University of Illinois Chicago, Chicago, Illinois, USA

[6]Research Histology and Tissue Imaging Core, University of Illinois Chicago, Chicago, Illinois, USA

Correspondence
Krista A. Varady and Lisa Tussing-Humphreys, Department of Kinesiology and Nutrition, University of Illinois Chicago, University of Illinois Cancer Center, 1919 W. Taylor St., Chicago, IL 60612, USA.
Email: varady@uic.edu and tussing@uic.edu

Funding information
National Institutes of Health, Grant/Award Numbers: U54MD012523, T32CA057699, R01DK119783, R01CA204808, R01CA250390, R01CA257807

Abstract

Objective: Approximately 42% of American adults are living with obesity, increasing their risk of colorectal cancer (CRC). Efficacious approaches to prevent and treat obesity may reduce CRC incidence. Daily calorie restriction (Cal-R) is the most common approach to treating obesity, yet clinically meaningful weight loss is elusive owing to waning adherence. Time-restricted eating (TRE) consists of consuming foods within a specified time frame, creating a natural calorie deficit. TRE in animals shows cancer protective effects. In humans, TRE is safe and acceptable among adults with obesity, producing ~3% to 5% weight loss and reductions in oxidative stress and insulin resistance. However, TRE has not been tested rigorously for CRC preventive effects.

Methods: The authors describe a 12-month randomized controlled trial of 8-hour TRE (ad libitum 12 PM-8 PM), Cal-R (25% restriction daily), or Control among 255 adults at increased risk for CRC and with obesity.

Results: Effects on the following will be examined: 1) body weight, body composition, and adherence; 2) circulating metabolic, inflammation, and oxidative stress biomarkers; 3) colonic mucosal gene expression profiles and tissue microenvironment; and 4) maintenance of benefits on body weight/composition and CRC risk markers.

Conclusions: This study will examine efficacious lifestyle strategies to treat obesity and reduce CRC risk among individuals with obesity.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2022 The Authors. *Obesity* published by Wiley Periodicals LLC on behalf of The Obesity Society.

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer and cause of cancer-related death among men and women in the United States (US) with an estimated 106,180 new cases and more than 52,000 deaths expected in 2022 [1]. The majority of CRC is sporadic, meaning the gene mutations are acquired rather than inherited [2]. Factors such as smoking, environmental toxicants, diet, and obesity are major drivers of sporadic CRC [1, 3]. Obesity, affecting 42% of US adults, is associated with metabolic, hormonal, and immune perturbations that can promote gene mutations that drive tumorigenesis [4, 5]. For every 5-unit increase in BMI (kg/m^2), CRC risk increases by approximately 5% [6]. Preventing and treating obesity could have significant effects on CRC incidence in the US.

Daily calorie restriction (Cal-R) is the most common approach to weight loss. Cal-R focuses on decreasing daily calorie intake by about 25% of kilocalorie needs for body weight maintenance through behavioral changes, including frequent self-monitoring of calories, food, and body weight. Cal-R can reduce body weight, decrease insulin resistance, and lower chronic inflammation, all of which are associated with the development of CRC [5, 7]. However, only a few studies have examined the effect of Cal-R on the colon tissue among individuals with obesity [8–10]. Among 10 participants with obesity, Pendyala et al. [9] found that medically supervised Cal-R was associated with reduced tissue inflammation (e.g., CD3, CD163) and downregulation of cancer-related gene pathways (e.g., signal transducer and activator of transcription, nuclear factor-κB). Similarly, in 20 adults with obesity, Beeken et al. [10] demonstrated that 8 weeks of medically supervised Cal-R was associated with decreased colonocyte proliferation (i.e., antigen Ki67). Magkos [8] and colleagues reported that traditional Cal-R decreased colonocyte proliferation rate in 17 adults with obesity. Although promising, the existing studies are small (between 10 and 20 participants), highly controlled, and short in duration. Clearly, there is a need for rigorous large-scale studies to decipher the colon tissue-specific mechanisms underlying the anticancer effects of Cal-R in adults with obesity.

Time-restricted eating (TRE), a form of intermittent fasting, is an approach to eating in which the overnight fasting period is extended. Individuals are instructed to limit food intake to a specified time frame, typically between 4 and 10 hours daily, and fast for the remainder the day. Recent data from small short-term studies (~12-16 weeks) have suggested that individuals are highly adherent to TRE (5.6 ± 0.3 d/wk over a 12-week period) and that they reduce daily calorie intake by ~300 to 500 kcal daily without calorie counting or food quality changes [11, 12]. Data have also suggested that TRE results in clinically meaningful weight loss and improvements in insulin sensitivity, systemic inflammation, oxidative stress, and circulating insulin-like growth factor I (IGF-I) and leptin [13–17]. Moreover, TRE reduces body fat, while retaining lean muscle mass [12, 17–19]. To our knowledge, there has been only one long-term study of TRE. This 12-month study examined TRE + 25% Cal-R versus daily 25% Cal-R [20]. Results showed comparable adherence, weight loss, and cardiometabolic benefit (i.e., fat mass, blood pressure, glucose, and lipid levels) between the two groups [20]. The effects of long-term TRE alone (i.e., without Cal-R) on weight loss and circulating and tissue-level CRC risk markers have not been rigorously investigated in persons with obesity, nor do we know whether TRE is superior or similar to Cal-R for mitigating CRC.

This paper describes the design of a TRE-CRC trial that will test the effect of 12 months of TRE compared with Cal-R on the primary outcome of body weight change. Secondary outcomes include body fat loss, circulating metabolic, inflammatory, and oxidative stress biomarkers, colonic mucosal gene expression profiles and colonic mucosal markers of inflammation, DNA damage, and cellular growth in adults with obesity who are at an elevated risk of CRC. We hypothesize that TRE participants will be more adherent to the intervention resulting in greater weight and body fat loss versus Cal-R/Control. For secondary outcomes, we hypothesize that TRE participants will experience greater effects for the following:

1. Improvements in circulating metabolic, proinflammatory, and oxidative stress biomarkers compared with Cal-R/Control, which will be negatively associated with percentage weight loss.

Study Importance

What is already known?

- Time-restricted eating (TRE) produces modest weight loss.
- TRE produces a natural caloric deficit by shortening the daily eating window.
- TRE has high adherence.

What does this study add?

- What is the effect of TRE compared with calorie restriction (Cal-R) on body weight, body composition, and intervention adherence, as well as maintenance of health benefits?
- What is the effect of TRE compared with Cal-R on circulating metabolic, inflammation, and oxidative stress biomarkers?
- What is the effect of TRE compared with Cal-R on colonic mucosal gene expression profiles and tissue microenvironment?

How might these results change the direction of research or the focus of clinical practice?

- These results will compare systemic and colon tissue molecular mechanisms that mediate the anticancer effects of both TRE and Cal-R.
- These data will demonstrate whether 8-hour TRE can be implemented as an alternative to traditional Cal-R for both weight loss, weight-loss maintenance, and CRC risk reduction in adults with obesity.

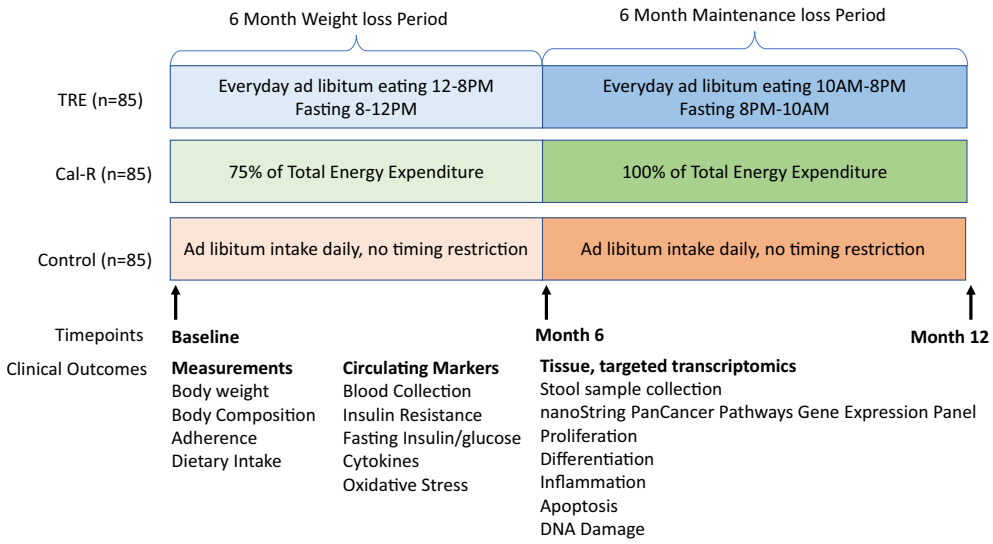

FIGURE 1 Trial design. Cal-R, daily calorie restriction; TRE, time-restricted eating [Color figure can be viewed at wileyonlinelibrary.com]

2. Downregulation of genes in pathways related to inflammation and proliferation and upregulation of genes in pathways related to apoptosis and DNA damage control that correlate with greater weight loss and weight-loss-related improvements in the circulating biomarkers compared with Cal-R/Control.
3. Decreases in mucosal markers of inflammation, DNA damage, and proliferation and increases in markers of differentiation and apoptosis that correlate with greater weight loss and weight-loss-related improvements in the circulating biomarkers and mucosal gene expression profiles.

METHODS

The experimental protocol was approved by the University of Illinois Chicago (IRB# 2020-0167). Participants will provide written informed consent prior to study participation. The trial is registered at ClinicalTrials.gov (NCT05114798).

Study design

This study is a 12-month (6-month intervention, 6-month maintenance), controlled, parallel-arm, randomized trial (Figure 1). A total of 255 adults with obesity (45-65 years old) who are at elevated CRC risk and who have prediabetes will be randomized 1:1:1 to one of three groups: 1) 8-hour TRE; 2) Cal-R; or 3) Control.

Setting and recruitment

A total of 255 individuals residing in the Chicagoland area will be recruited and enrolled. Recruitment will take place through the University of Illinois Health Gastrointestinal Lab as well as via social media, online media, and Researchmatch.org. We will partner with University of Illinois Health to conduct the trial. Data collection visits will take place at the University of Illinois Chicago. The intervention sessions will take place in person or remotely based on participant preference.

Participants

Screening

Individuals will initially be screened by phone. If the individual meets the basic enrollment criteria, they will be invited to an in-person screening. Written informed consent will be obtained prior to initiating the in-person screening. Individuals will be weighed using an electronic scale and height will be assessed with a fixed stadiometer to confirm BMI (kilograms per meters squared). A fasting (12 hour) venous blood draw will be performed to determine glycated hemoglobin and serum glucose and insulin. Participants will be instructed to keep a 7-day food record starting the next day. A remote follow-up screening visit will occur within 8 to 10 days, during which lab results and completeness of the food record will be discussed. Individuals meeting all screening metrics will be invited to participate in the full trial (Table 1).

Inclusion

Inclusion criteria is as follows: being 45 to 65 years of age, having BMI of 30 to 49.99 kg/m^2, being at an elevated risk of CRC (≥1 adenomatous polyp or an adenomatous polyp > 1 cm in the previous 5 years), being up to date with CRC screening/surveillance, having prediabetes, and the ability to complete a 7-day food record.

TABLE 1 Inclusion and exclusion

Inclusion criteria	Exclusion criteria
Age: 45-65 years	History of chronic disease
BMI: 30-49.99 kg/m^2	Type 1 or 2 diabetes
Elevated CRC risk	Cancer treatment within the past 12 months
Current with colorectal cancer screening/ surveillance	Severe mental health disorder
Current prediabetes	History of eating disorder or bariatric surgery
Able to complete a 7-day food record	Alcohol abuse, illicit drug use, or smoking combustable tobacco
	Currently on weight-loss diet/program or not weight stable for 3 months
	Anticoagulant medications, medications with endoscopic risk, antivirals, or immunosuppressants
	Night shift workers
	Pregnant or trying to become pregnant
	Perimenopausal or irregular menstrual cycles

Abbreviation: CRC, colorectal cancer.

Exclusion

Participants will be excluded if they have a history of chronic disease (e.g., renal disease, autoimmune disorders, malabsorptive disorders, gastrointestinal or hepatic disease, severe ischemic heart disease), type 1/2 diabetes or undiagnosed uncontrolled diabetes, cancer treatment in the past 12 months, severe mental health disorder, history of eating disorder, or bariatric surgery. Participants will be excluded for the following: alcohol intake > 50 g/d, use of illicit drugs, or use of combustible tobacco. Participants who are on a weight-loss diet or involved in a formal weight-loss program or are not weight stable for 3 months (±4.5 kg) prior to the study will be excluded. Individuals taking drugs that may influence study outcomes (weight loss), anticoagulant medications or medications with endoscopic risk and antivirals or immunosuppressant medications, will be excluded from the study. Night shift workers will also be excluded. Last, females who are pregnant, who are trying to become pregnant, who are perimenopausal, or who have irregular menstrual cycles (must have absence of menses for >2 years or regular menstrual cycle between 27 and 32 days) will be excluded from the study.

Randomization

Participants will be randomized by the data manager by way of a stratified random sample in a 1:1:1 ratio. The sample frame will be divided into strata based on BMI (30-39.99 and 40-49.99), sex, and age (45-54 and 55-65 years old). Participants from each stratum will then be randomized to one of three groups: 1) TRE; 2) Cal-R; or 3) Control.

Intervention

Design

This study is a 12-month, controlled, parallel-arm trial comparing the effects of TRE versus daily Cal-R versus a no-intervention control. The 12-month study will be split into a 6-month weight-loss phase and a 6-month weight-maintenance phase. During the first 6 months, participants will meet with the study team weekly and biweekly thereafter. Meetings will take place in person and remotely with participant preference in mind. All participants will be instructed to maintain their current level of physical activity throughout the duration of the trial.

Intervention arms

TRE

Weight-loss phase. During the active weight-loss phase, participants in the TRE group will be instructed to eat ad libitum from 12 PM to 8 PM daily and to fast from 8 PM to 12 PM daily (Figure 2). Previous work in intermittent fasting has suggested that placing meals around dinner improves adherence as individuals are able to eat with their families and maintain social obligations [21]. During the 8-hour eating window, participants will not be required to monitor calories or restrict types or quantities of foods. Water and calorie free beverages (black coffee, tea, diet soda, etc.) will be permitted during the fasting period. Only a total of two diet sodas will be permitted each day, as it is possible that they can increase sugar cravings [22]. TRE participants will meet with a TRE interventionist for 30 minutes at the start of the intervention to review instructions and goals and continue to meet every week throughout the 6-month weight-loss period.

Weight-maintenance phase. Individuals will be instructed to maintain their body weight during the weight-maintenance phase. Individuals will extend the TRE window to 10 hours daily (10 AM-8 PM). Participants will continue to meet biweekly with the TRE interventionist.

Adherence. At the same day and time each week, participants will be asked to transmit a picture of their weight from a home scale via a secure text-messaging platform. Each day, participants will respond to a text message to indicate their eating start and stop time. If the message indicates that the participant ate only within the prescribed eating window (±30 minutes of 12 PM and 8 PM), that day will be labeled "adherent." If the message indicates that the participant consumed food outside the prescribed window, that day will be labeled "non-adherent." Adherence to the TRE intervention will be assessed as the number of adherent days per week.

Cal-R

Weight-loss phase. During the active weight-loss phase, participants in the Cal-R group will restrict energy intake by 25% of their total energy expenditure daily (Figure 2). Total energy expenditure will be calculated by indirect calorimetry (MedGem Indirect Calorimeter, Microlife USA),

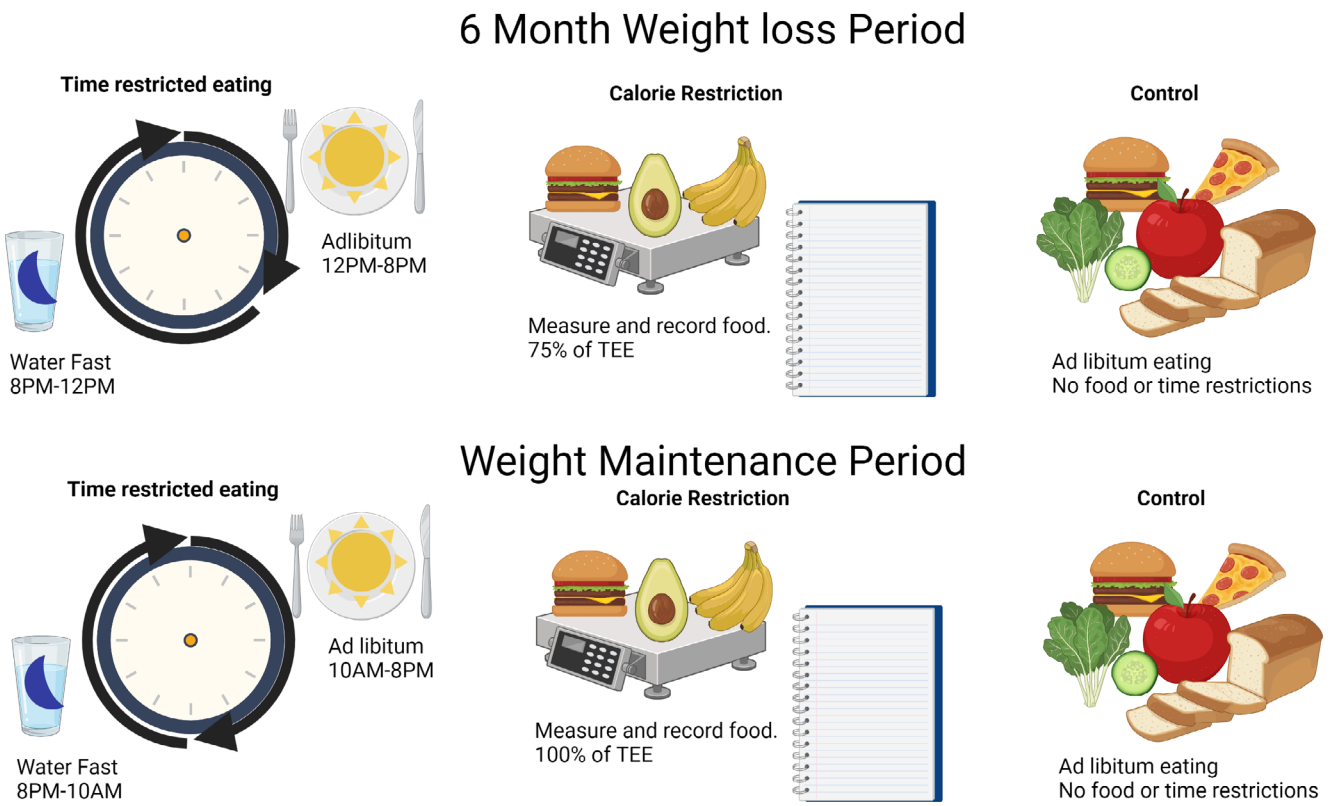

FIGURE 2 12-month diet intervention. TEE, total energy expenditure [Color figure can be viewed at wileyonlinelibrary.com]

which will produce a resting metabolic rate (RMR). The resting metabolic rate estimation will then be multiplied by an activity factor related to the participants' usual physical activity level. Cal-R participants will meet with the Cal-R interventionist weekly during the first 6 months.

Weight-maintenance phase. At the start of the weight-maintenance phase, energy needs will be reevaluated using indirect calorimetry. Individuals will be instructed to consume 100% of energy needs to maintain body weight. Participants will meet biweekly with the Cal-R interventionist.

Adherence. At the same day and time each week, participants will be asked to transmit a picture of their weight from a home scale via a secure text-messaging platform. Each month, participants will complete a 7-day food record. Energy intake will be estimated from the food records and compared with the calorie recommendation provided.

Control

Control participants will not receive dietary counseling. Control participants will be offered their choice of the TRE or Cal-R intervention following completion of the 12-month data collection visit.

Intervention fidelity

To assure fidelity, intervention sessions will be semistructured in a manualized format. Given the individualized nature of the sessions, the interventions cannot be fully scripted. A semistructured approach will allow for use of a fidelity checklist itemizing the expected general content for sessions. The interventionist will record all sessions, and non-interventionist staff will randomly select an active and maintenance session from each intervention arm and complete the fidelity checklist. Senior staff will meet with the interventionists to provide feedback from the fidelity checklist and to address deviations from the intended intervention content.

Data collection and measures

Data collection will occur at baseline and at months 3, 6, 9, and 12. The following measures will be collected at these time points unless stated otherwise.

Body weight and body composition

Body weight will be measured in a fasted state to the nearest 0.1 kg using a calibrated digital scale (Tanita BWB-800). Body composition will be measured by dual energy x-ray absorptiometry (iDXA, GE Healthcare) at baseline, month 6, and month 12 only. Height will be measured at screening using a fixed stadiometer (Seca). BMI will be calculated as weight (kilograms)/height (meters squared) from screening height and measured body weights.

Dietary intake

Dietary intake will be measured via 7-day food records. Participants will be asked to measure the portion amounts of foods consumed with household measures (e.g., measuring cups), as well to record the timing of food intake. Food records will be distributed as paper records and then will be entered by study staff into Automated Self-Administered Dietary Assessment tool - 24. Participants will be given instruction on how to keep an accurate and complete food record, a sample completed food record for reference, and portion size estimates. The study coordinator will review the participant food records for completeness. The Diet History Questionnaire III, a validated food frequency questionnaire, will be administered at baseline and at 12 months only to determine habitual intake [23, 24].

Physical activity

Physical activity will be quantified using a validated pattern recognition monitor (GENEActiv, Activeinsights, Ltd.). Participants will wear the monitor on their wrist for 7 days. The activity monitor integrates motion data from a triaxial accelerometer. The data will be processed using the company's open-source software and code. The purpose of accelerometry data is to determine changes in physical activity and sleep behaviors.

Circulating biomarkers

Fasting (12 hour) blood samples will be obtained at baseline and at months 6 and 12 and processed for plasma and serum; blood will be stored at −80 °C or shipped for analysis. Glycated hemoglobin and serum glucose and insulin will be analyzed by a local commercial lab (Quest Diagnostics). Insulin resistance will be calculated using the following homeostasis model assessment (HOMA) method: (HOMA-IR = fasting insulin [microinsulin units/milliliter] × fasting glucose [milligrams/deciliter]/405). Plasma IGF-I, adipokines (adiponectin and leptin), cytokines (tumor necrosis factor-α, interleukin [IL]-6, IL1β, and IL-10), and oxidative stress biomarkers (8-isoprostane, 4-hydroxynonenal adducts, protein carbonyls, and nitrotyrosine) will be measured by immunoassay (R&D Systems; Cayman Chemica; Cell BioLabs). All samples will be measured in duplicate by a lab technician blinded to treatment.

Colonic mucosa sampling

Healthy colonic mucosa will be obtained at baseline and at months 6 and 12. An unprepped limited flexible sigmoidoscopy will be performed to obtain four "double-bite" tissue biopsies (1- to 2-mm small pinhead-sized tissue pieces). This procedure will be limited to the last 20 to 25 cm of the large intestine. The tissue will be placed in RNAlater (Invitrogen, ThermoFisher Scientific), held for 24 hours at 4 °C, and then frozen at −80 °C until analysis. For histology, fresh tissue will be placed in 10% neutral buffered formalin (Sigma Aldrich), held at 4 °C for 12 hours, and transferred to the Research Histology and Tissue Imaging Collaborative facility, for further processing and analysis.

Targeted tissue gene expression

Total RNA will be extracted from tissue using the using Maxwell RSC simplyRNA Tissue Kit with the Maxwell RSC Instrument (Promega). Prior to gene expression analysis, RNA quality and quantity will be determined using TapeStation4200 (Agilent Technologies) and Qubit Fluorimeter (ThermoFisher). Gene expression profiling will be conducted on the nCounter Sprint platform (NanoString) using the PanCancer Pathways panel, a multiplex gene expression analysis of 770 genes representing 13 canonical pathways in cancer. Barcoded tissue RNA will be analyzed using kits, reagents, and methods per the manufacturer's instructions. NanoString nSolver and Rosalind (Rosalind, Inc.) software will be used to analyze the nCounter-generated data. *P* values and fold change values for genes, accounting for background signals in negative controls and normalization factors derived from housekeeping genes and positive controls, will be examined.

Immunohistochemistry

Nine sections will be cut from each paraffin-embedded tissue sample. One section will be stained with hematoxylin and eosin. Remaining sections will be immunostained for markers of proliferation (Ki67), inflammation (CD3, CD163, phospho [p]-IκB kinase [IKK] a/b), apoptosis (c-caspase-3, BCL-2 associated X protein [Bax]), differentiation (p21), and DNA damage (8-OHdG). Staining, including the antigen retrieval step and hematoxylin counterstain, will be performed on a BOND RX autostainer (Leica Microsystems) using BOND Polymer Refine Detection kit (Leica Biosystems, #DS9800), Bond Epitope Retrieval Solution 1 (pH 6, Leica Microsystems, #AR9961), or Bond Epitope Retrieval Solution 2 (pH 9, Leica Microsystems, #AR9640). Suitable positive and negative controls will be included in each staining run, and samples from the three treatment groups will be balanced in each run to minimize bias due to inter-run variance. All slides will be scanned at 20× on a Leica AT2 high-throughput scanner. In each whole slide image, regions of interest (colonic crypt areas) will be identified by combining machine learning and manual drawing using HALO image analysis software (Indicalabs). Nuclear markers (Ki67, p21, 8-OHdG) will be quantified using HALO as a percentage of positive cells within the total epithelial cells in the region. For markers that could be differentially expressed based on crypt level (Ki67, p21), in addition to whole crypt analysis, a random sample of crypts from each biopsy will be divided in half from base to lumen and positive cells

counted in the upper and lower half. Immune cell markers will be quantified based on their density within a peri-epithelial zone, and the metric for cytoplasmic or diffuse markers such as Bax, c-caspase-3, and p-IKKa/b will be positive area over total epithelial area. Analysts will be blinded to treatment assignment.

Adverse event monitoring

Follow-up by phone will occur within 48 hours of the flexible sigmoidoscopy procedure to evaluate procedure-related health status changes and adverse events. Intervention-related adverse events will be monitored and documented weekly through a REDCap survey link. Events will be classified according to a standardized coding scheme [25].

Covariates that could influence adherence and treatment effects

At baseline, participant sociodemographic status and health history will be assessed. At all in-person data collection visits, mental health (symptoms of anxiety [Generalized Anxiety Disorder-7], stress [Perceived Stress Scale], depression [Patient Health Questionnaire-8], body image [Body Shape Questionnaire], self-efficacy, disinhibited eating [Three Factor Eating Questionnaire], perceived social support) [26–32], sleep hygiene (Pittsburgh Sleep Quality Index) [33], and medication/supplement use will be determined. We will also obtain the participant's most recent colonoscopy report (obtained at screening) to characterize colorectal adenoma type(s), number, location, and size.

Power and sample size

The power and sample size calculations are based on the primary outcome measure, percentage change in body weight from baseline to post-intervention. We estimated that the TRE group would lose ~8% and the Cal-R group would lose ~4% of baseline body weight over 6 months, versus Control participants [11, 12]. The overall significance level of three pairwise comparisons is controlled at 0.05 so that the adjusted type I error for each pairwise comparison is set at 0.0167 according to Bonferroni correction. The minimum sample is determined by the comparison between TRE and Cal-R, which has the smallest difference and biggest standard deviation. The minimum required sample size is 64 participants per group to achieve at least 90% power for detecting a significant difference in body weight of any pairwise comparison among the TRE, Cal-R, and Control groups at post-intervention using a two-sided, two-sample, unequal-variance t test at $\alpha = 0.0167$. Based on dropout data from previous TRE and intermittent fasting studies, we anticipate a dropout rate of ~25% [34]. Thus, we initially aim to recruit 255 participants (85 per group), assuming 192 (64 per group) will complete the trial. Significance levels for secondary outcomes will not be adjusted for multiple comparisons or number of secondary outcomes. With 64 participants per group, minimum detectable mean changes are calculated at the significance level of 0.05 and a power of ≥90% to detect circulating and tissue-based secondary outcomes at month 12.

Data management

A large majority of data will be recorded directly or later uploaded into REDCap, which is a secure web-based platform for building and managing online research-related databases and surveys [33].

DATA ANALYTIC PLAN

Data will be analyzed using the statistical software SAS version 9.4 (SAS Institute Inc.). Both intent-to-treat and per-protocol analyses will be completed. The per-protocol analysis set includes all enrolled participants who receive at least some of the treatment and who do not have major treatment deviations. To achieve the goal of a well conducted clinical trial according to Good Clinical Practice, every effort will be made to collect all data; however, missing or incomplete data may be reported. All missing or partial data will be presented in the patient data listing as they are recorded and determined for the nature of their absence (missing completely at random, missing at random, or systematic missing). Unretrievable missing values will be replaced by values computed with statistically appropriate models (e.g., linear model, Bayesian bootstrap methods). Multiple imputation analysis will be conducted to address missing data.

Summary statistics will be estimated for all variables. Continuous variables will be examined for distributions and the presence of outliers. Variables that are not normally distributed will be transformed; if normality cannot be achieved, they will be analyzed using nonparametric tests. Binary or categorical variables will be summarized with frequencies and percentages. In the primary analyses, change in body weight from baseline to months 6 and 12 will be estimated among each of the three groups, and 95% confidence intervals (CIs) will be constructed assuming normal distribution, respectively. Two-sample two-sided t tests will be employed to compare the change in body weight in each pairwise comparison among the three groups. General linear models will be employed in the multivariable analysis to estimate the difference in body weight change between the three groups after adjusting for other factors, including covariates that could influence adherence and treatment effects. Mixed models will be used to compare the longitudinal data of body weight, BMI, body fat mass and distribution, dietary adherence, and energy restriction over the intervention period.

Similarly, the secondary continuous outcomes, such as the changes in insulin resistance (HOMA-IR), fasting insulin/glucose, cytokines, oxidative stress (e.g., 8-isoprostane), colonocyte proliferation (Ki67), and colonocyte markers of apoptosis (c-caspase-3, Bax), differentiation (p21), and inflammation (CD3, CD163, p-IKKa/b), will be estimated among each of the three groups, and 95% CIs will be constructed assuming normal distribution. A two-sample t test will then be used to perform pairwise comparisons among the three groups. Generalized

linear models will be employed to estimate the adjusted difference in each of the secondary continuous end points between the three groups after adjusting for other factors, including covariates that could influence adherence and treatment effects. For binary secondary end points, such differences will be compared using a Fisher exact test or χ^2 test. Logistic regression will be further employed to compare these binary end points between the three groups after adjusting for other factors. Odds ratios and 95% CIs will be calculated to evaluate the strength of any association that emerged. We will also examine correlations between weight loss and circulating and tissue-specific biomarkers with Pearson or Spearman correlation coefficients and tested by Wald's test.

Genes identified as having a p value ≤ 0.05 and fold change $> \pm 1.5$ will be evaluated and reported based on statistical and biological significance. Differentially expressed gene-associated pathways will be reported and separated into groups of unregulated or downregulated genes, then subjected to gene ontology enrichment analysis to identify associated biological processes. NanoString-identified gene and pathway scores will be analyzed for potential associations with degree of weight loss and change in the circulating biomarkers via linear regression. A two-sample t test will be used to compare the expression of genes in pathways related to inflammation and proliferation as well as the genes in pathways related to apoptosis and DNA damage control between TRE and Cal-R/Control arms.

DESIGN CONSIDERATIONS

Participant retention

All participants will receive a monetary subsidy for participating in the trial. For TRE and Cal-R participants, to maximize retention, we will provide support for transportation and offer videoconferencing when appropriate. We will also accommodate for major holidays (e.g., Thanksgiving Day) allowing for an "off" day. For Control participants, we will provide participants with a transportation subsidy to visit the campus for research assessments. Control participants will be provided 3 months of free dietary weight-loss counseling after study completion.

Participant safety

If a colon polyp is visualized during the research flexible sigmoidoscopy, the participant will be referred for a fully prepped colonoscopy. The healthy mucosal biopsy will be sampled a minimum of 5 cm from the visualized polyp(s) to minimize potential field effect.

Field effect in persons with history of colorectal adenoma

Genetic alterations occur in fields of precancerous cells, including colorectal adenoma and aberrant DNA methylation, and may play an important role in field cancerization [35]. Methylation of the O^6-methylguanine-DNA methyltransferase (MGMT) gene promoter is a commonly observed field effect in up to 40% of persons with colorectal adenoma [36]. Field effect in what is otherwise healthy colonic mucosa can impact tissue gene expression and other molecular features. It is also possible that TRE and Cal-R could have a differential effect in participants without tissue field effects compared with participants with field effects [35]. To account for field effect, we will measure MGMT promoter methylation in healthy colonic mucosa at baseline by extracting DNA from tissue (DNeasy, Qiagen) and conducting methylation-specific polymerase chain reaction (PCR) [37].

Identified a priori limitations

TRE and Cal-R show similar effects on body weight and circulating and tissue-level markers

It is possible that TRE and Cal-R will have similar effects. This finding would still be significant and informative from a clinical and public health perspective given that it would provide evidence that both TRE and Cal-R are efficacious for weight loss and CRC risk reduction among individuals with obesity, while providing comprehensive insight regarding their mechanistic underpinnings. The only yearlong study of TRE reported similar findings between TRE and Cal-R; however, both groups were asked to count and monitor intake [20]. Because weight loss and cardiometabolic risk were not significantly different, this group postulated that the weight and cardiometabolic risk are from caloric restriction alone [20]. We will not be asking the TRE group to reduce calorie intake or self-monitor food intake. If participants obtain the same weight loss and decreases in CRC risk-related markers as Cal-R, by just watching the clock, this finding could be significant in reducing the burden of behavioral change associated with poor adherence to Cal-R, while still decreasing weight and disease risk.

Dietary-pattern effects on circulating and tissue-level markers

It is possible that dietary factors or diet quality influence the circulating and tissue-level markers of interest. We plan to rigorously assess dietary intake behaviors through multiple 7-day food records and the Diet History Questionnaire III. There are limitations to the dietary food records, including potential for reactivity and underreporting; however, we are providing instruction and reviewing all food records for completeness and accuracy, and it is an important method to determine dietary intake changes [38, 39]. Analyses of interactions between baseline dietary factors and treatment effects will be explored to address the heterogeneity of dietary features such as percentage of calories from different macronutrient categories or vegetarian versus non-vegetarian diet patterns.

Other mechanisms mediating TRE response on CRC risk

Although our approach is comprehensive, we fully acknowledge that other pathways and systems could be potential mediators of TRE response, including synchronization with the circadian clock, autophagy, and its effects on gut microbiome [40, 41]. We intend to optimize the experimental design and leverage the infrastructure to collect additional samples (e.g., stool) as financially feasible so that our team is well positioned to investigate additional pathways and systems through which TRE, as well as Cal-R, mitigates CRC risk. Additionally, the flexible sigmoidoscopy will only sample tissue from the sigmoid colon, and it is known that tumors emerging from the left and right colon may have their own risk factors, molecular features, microbiome distribution, and prognosis [42].

DISCUSSION

In 2020, the National Cancer Institute (NCI) released a request for applications to address nine "provocative" questions relevant to cancer research [43]. One of the provocative questions posed was "How does intermittent fasting affect cancer incidence, treatment response, or outcome?" This call was predicated, to some extent, on the strong preclinical data showing prolonged daily calorie restriction and intermittent fasting in animals having positive effects on cancer incidence [5, 40, 41]. Specifically, the NCI was requesting proposals that took a transdisciplinary approach to understanding underlying mechanisms through which intermittent fasting approaches such as TRE impart a cancer protective effect. Moreover, the NCI was interested in applications that addressed intermittent fasting's effect on cancer risk factors (e.g., body weight) and an individual's adherence to the intermittent fasting regimen versus traditional approaches to weight loss (i.e., Cal-R). The TRE-CRC trial will serve as the first study, to our knowledge, of TRE efficacy for weight loss and CRC risk reduction in adults with obesity who are also at elevated CRC risk. The study will demonstrate whether 8-hour TRE can be implemented as an alternative to traditional Cal-R for both weight loss, weight-loss maintenance, and CRC risk reduction. The TRE-CRC study will also be, to our knowledge, the first and largest study in humans to comprehensively examine and compare systemic and colon tissue molecular mechanisms that mediate the anticancer effects of both TRE and Cal-R. Approximately 42% of American adults are living with obesity [44], increasing their risk of CRC by approximately 30% [45]. Our study is addressing a critical need for efficacious lifestyle strategies to treat obesity and reduce CRC risk among individuals with obesity.O

FUNDING INFORMATION

This work was supported by NIH R01CA257807 (Lisa Tussing-Humphreys, and Krista A. Varady), R01CA250390 (Lisa Tussing-Humphreys, Marian L. Fitzgibbon), R01CA204808 (Lisa Tussing-Humphreys), R01DK119783 (Krista A. Varady), T32CA057699 (Andrew McLeod), U54MD012523 (Lisa Tussing-Humphreys). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

CONFLICT OF INTEREST

Krista Varady is the author of the book "The Every Other Day Diet" published by the Hachette Book Group. The other authors declared no conflict of interest.

CLINICAL TRIAL REGISTRATION

ClinicalTrials.gov identifier NCT05114798.

ORCID

Kelsey Gabel https://orcid.org/0000-0003-4613-8892
Krista A. Varady https://orcid.org/0000-0002-9279-9934

REFERENCES

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin*. 2022;72:7-33.
2. Carethers JM, Jung BH. Genetics and genetic biomarkers in sporadic colorectal cancer. *Gastroenterology*. 2015;149:1177-1190.e3.
3. de la Chapelle A. Genetic predisposition to colorectal cancer. *Nat Rev Cancer*. 2004;4:769-780.
4. Stone TW, McPherson M, Gail DL. Obesity and cancer: existing and new hypotheses for a causal connection. *EBioMedicine*. 2018;30:14-28.
5. Longo VD, Fontana L. Calorie restriction and cancer prevention: metabolic and molecular mechanisms. *Trends Pharmacol Sci*. 2010;31:89-98.
6. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet*. 2008;371:569-578.
7. Hursting SD, Dunlap SM, Ford NA, Hursting MJ, Lashinger LM. Calorie restriction and cancer prevention: a mechanistic perspective. *Cancer Metab*. 2013;1:10. doi:10.1186/2049-3002-1-10
8. Magkos F, Sullivan S, Fitch M, et al. Effect of weight gain and weight loss on in vivo colonocyte proliferation rate in people with obesity. *Obesity (Silver Spring)* 2017;25(suppl 2):S81-S86.
9. Pendyala S, Neff LM, Suárez-Fariñas M, Holt PR. Diet-induced weight loss reduces colorectal inflammation: implications for colorectal carcinogenesis. *Am J Clin Nutr*. 2011;93:234-242.
10. Beeken RJ, Croker H, Heinrich M, et al. The impact of diet-induced weight loss on biomarkers for colorectal cancer: an exploratory study (INTERCEPT). *Obesity (Silver Spring)* 2017;25(suppl 2):S95-S101.
11. Cienfuegos S, Gabel K, Kalam F, et al. Effects of 4- and 6-h time-restricted feeding on weight and cardiometabolic health: a randomized controlled trial in adults with obesity. *Cell Metab* 2020;32:366-378.
12. Gabel K, Hoddy KK, Haggerty N, et al. Effects of 8-hour time restricted feeding on body weight and metabolic disease risk factors in obese adults: a pilot study. *Nutr Healthy Aging*. 2018;4:345-353.
13. Gabel K, Varady KA. Current research: effect of time restricted eating on weight and cardiometabolic health. *J Physiol*. 2022;600:1313-1326.
14. Varady KA, Cienfuegos S, Ezpeleta M, Gabel K. Cardiometabolic benefits of intermittent fasting. *Annu Rev Nutr*. 2021;41:333-361.
15. Sutton EF, Beyl R, Early KS, Cefalu WT, Ravussin E, Peterson CM. Early time-restricted feeding improves insulin sensitivity, blood pressure, and oxidative stress even without weight loss in men with prediabetes. *Cell Metab*. 2018;27:1212-1221.
16. Jamshed H, Beyl RA, Della Manna DL, Yang ES, Ravussin E, Peterson CM. Early time-restricted feeding improves 24-hour glucose levels and affects markers of the circadian clock, aging, and autophagy in humans. *Nutrients*. 2019;11:1234. doi:10.3390/nu11061234
17. Moro T, Tinsley G, Bianco A, et al. Effects of eight weeks of time-restricted feeding (16/8) on basal metabolism, maximal strength, body composition, inflammation, and cardiovascular risk factors in

resistance-trained males. *J Transl Med*. 2016;14:290. doi:10.1186/s12967-016-1044-0
18. Tinsley GM, Forsse JS, Butler NK, et al. Time-restricted feeding in young men performing resistance training: a randomized controlled trial. *Eur J Sport Sci*. 2017;17:200-207.
19. McAllister MJ, Pigg BL, Renteria LI, Waldman HS. Time-restricted feeding improves markers of cardiometabolic health in physically active college-age men: a 4-week randomized pre-post pilot study. *Nutr Res*. 2020;(75):32-43.
20. Liu D, Huang Y, Huang C, et al. Calorie restriction with or without time-restricted eating in weight loss. *N Engl J Med* 2022;386:1495-1504.
21. Hoddy KK, Kroeger CM, Trepanowski JF, Barnosky A, Bhutani S, Varady KA. Meal timing during alternate day fasting: impact on body weight and cardiovascular disease risk in obese adults. *Obesity (Silver Spring)*. 2014;22:2524-2531.
22. Hill SE, Prokosch ML, Morin A, Rodeheffer CD. The effect of non-caloric sweeteners on cognition, choice, and post-consumption satisfaction. *Appetite*. 2014;83:82-88.
23. Kristal AR, Kolar AS, Fisher JL, et al. Evaluation of web-based, self-administered, graphical food frequency questionnaire. *J Acad Nutr Diet*. 2014;114:613-621.
24. Subar AF, Thompson FE, Kipnis V, et al. Comparative validation of the Block, Willett, and National Cancer Institute food frequency questionnaires: the Eating at America's Table Study. *Am J Epidemiol* 2001;154:1089-1099.
25. Brown EG, Wood L, Wood S. The medical dictionary for regulatory activities (MedDRA). *Drug Saf*. 1999;20:109-117.
26. Spitzer RL, Kroenke K, Williams JB, Lowe B. A brief measure for assessing generalized anxiety disorder: the GAD-7. *Arch Intern Med*. 2006;166:1092-1097.
27. Cohen S. Perceived stress in a probability sample of the United States. The social psychology of health. In: Spacapan S, Oskamp S, eds. *The Claremont Symposium on Applied Social Psychology*. Sage Publications, Inc.; 1988:31-67.
28. Kroenke K, Strine TW, Spitzer RL, Williams JB, Berry JT, Mokdad AH. The PHQ-8 as a measure of current depression in the general population. *J Affect Disord*. 2009;114:163-173.
29. Taylor MJ, Cooper PJ. Body size overestimation and depressed mood. *Br J Clin Psychol*. 1986;25:153-154.
30. Sallis JF, Grossman RM, Pinski RB, Patterson TL, Nader PR. The development of scales to measure social support for diet and exercise behaviors. *Prev Med*. 1987;16:825-836.
31. Stunkard AJ, Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *J Psychosom Res*. 1985;29:71-83.
32. Dahlem NW, Zimet GD, Walker RR. The multidimensional scale of perceived social support: a confirmation study. *J Clin Psychol*. 1991;47:756-761.
33. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res*. 1989;28:193-213.
34. Trepanowski JF, Kroeger CM, Barnosky A, et al. Effect of alternate-day fasting on weight loss, weight maintenance, and cardioprotection among metabolically healthy obese adults: a randomized clinical trial. *JAMA Intern Med*. 2017;177:930-938.
35. Giovannucci E, Ogino S. DNA methylation, field effects, and colorectal cancer. *J Natl Cancer Inst*. 2005;97:1317-1319.
36. Shen L, Kondo Y, Rosner GL, et al. MGMT promoter methylation and field defect in sporadic colorectal cancer. *J Natl Cancer Inst* 2005;97:1330-1338.
37. Oliver JA, Ortiz R, Melguizo C, Alvarez PJ, Gomez-Millan J, Prados J. Prognostic impact of MGMT promoter methylation and MGMT and CD133 expression in colorectal adenocarcinoma. *BMC Cancer*. 2014;14:511. doi:10.1186/1471-2407-14-511
38. Ortega RM, Perez-Rodrigo C, Lopez-Sobaler AM. Dietary assessment methods: dietary records. *Nutr Hosp*. 2015;31:38-45.
39. Lichtman SW, Pisarska K, Berman ER, et al. Discrepancy between self-reported and actual caloric intake and exercise in obese subjects. *N Engl J Med* 1992;327:1893-1898.
40. Longo VD, Mattson MP. Fasting: molecular mechanisms and clinical applications. *Cell Metab*. 2014;19:181-192.
41. Longo VD, Panda S. Fasting, circadian rhythms, and time-restricted feeding in healthy lifespan. *Cell Metab*. 2016;23:1048-1059.
42. Benedix F, Kube R, Meyer F, Schmidt U, Gastinger I, Lippert H. Comparison of 17,641 patients with right- and left-sided colon cancer: differences in epidemiology, perioperative course, histology, and survival. *Dis Colon Rectum*. 2010;53:57-64.
43. National Institutes of Health, National Cancer Institute. Research answers to National Cancer Institute's (NCI) provocative questions (R01 clinical trial optional). Published January 17, 2020. Accessed September 12, 2022. https://grants.nih.gov/grants/guide/rfa-files/rfa-ca-20-004.html
44. Centers for Disease Control and Prevention. *Adult obesity facts*. Updated May 17, 2022. Accessed September 12, 2022. https://www.cdc.gov/obesity/data/adult.html.
45. World Cancer Research Fund, American Insitute for Cancer Research. Continuous update project. Diet, nutrition, physical activity and colorectal cancer. Published 2017. Revised 2018. Accessed September 12, 2022. https://www.wcrf.org/wp-content/uploads/2021/02/Colorectal-cancer-report.pdf

How to cite this article: Gabel K, Fitzgibbon ML, Yazici C, et al. The basis and design for time-restricted eating compared with daily calorie restriction for weight loss and colorectal cancer risk reduction trial (TRE-CRC trial). *Obesity (Silver Spring)*. 2022;30(12):2376-2385. doi:10.1002/oby.23579