Title
Effects of time-restricted eating on weight loss and other metabolic parameters in women and men with overweight and obesity: the TREAT study, a randomized controlled trial

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Effects of time-restricted eating on weight loss and other metabolic parameters in women and men with overweight and obesity: the TREAT study, a randomized controlled trial.

by

Dylan Lowe

DISSERTATION

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Dedication and Acknowledgments

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Effects of time-restricted eating on weight loss and other metabolic parameters in women and men with overweight and obesity: the TREAT study, a randomized controlled trial

Dylan A. Lowe

Abstract

IMPORTANCE Time-restricted eating has increased in popularity among people trying to lose weight. However, the efficacy and safety have not been explored in a large, prospective randomized trial.

OBJECTIVE To compare the effects of weight loss in people who are overweight and obese prescribed a time-restricted eating protocol versus standard three meals per day.

DESIGN, SETTING, AND PARTICIPANTS The Time-Restricted Eating (TREAT) study was a 12-week randomized controlled trial of men and women aged 18-64 with a body mass index of 27-43 kg/m^2. The study was conducted on a custom mobile study app on the Eureka Research Platform, and participants received a Bluetooth scale for daily use. Participants lived anywhere in the United States, with a subset of participants (n=50) living within 60 miles of San Francisco who underwent comprehensive, in-person metabolic testing.

INTERVENTION Subjects were randomized to one of two eating plans and received daily reminders about their eating windows through the study app. The consistent meal timing (CMT) group was instructed to eat three structured meals per day. The time-restricted eating (TRE) group was instructed to eat *ad libitum* from 12:00 pm until 8:00 pm and completely abstain from caloric intake from 8:00 pm until 12:00 pm the following day (16h fast:8h eat). Specific recommendations for caloric intake or macronutrient content were not prescribed to either group, and participants were not advised to make any changes to their physical activity.

MAIN OUTCOMES AND MEASURES The primary outcome was weight loss as measured at home on the Bluetooth-connected scale. Secondary outcomes were weight loss in the in-person cohort and changes in fat mass, lean mass, fasting insulin, fasting glucose, HbA1C, total energy expenditure, and resting energy expenditure. Bonferroni-corrected alphas and confidence intervals were used to correct for multiple comparisons for secondary outcomes. All other outcomes were considered exploratory and were not corrected for multiple outcomes.

RESULTS 116 subjects (mean [SD] age, 46.5 [10.5] years), participated in the study. There was a significant decrease in weight in the TRE (-0.94kg, 95% CI, -1.68, -0.20, p=0.013), but no significant change in the CMT group (-0.68kg, 95% CI, -1.41, 0.05, p=0.07) or between groups (-0.26kg, 95% CI, -1.30, 0.78, p=0.63). In the in-person cohort, (n=25 TRE, n=25 CMT), there was a significant within-group decrease in weight in the TRE group (-1.70kg, 95% CI, -2.56, -0.83, p<0.001), but no significant changes in any of the other secondary outcomes. There was a significant within-group decrease in lean mass in the TRE group (-1.10kg, 95% CI, -1.73, -0.48, p<0.001). There was also a significant difference in appendicular lean mass index between groups (-0.162kg/m2, 95% CI, -0.274, -0.050, p=0.005).

CONCLUSIONS AND RELEVANCE Prescribing TRE to people who are overweight and obese led to modest weight loss, but there was no difference in weight loss when compared to those randomized to CMT. Moreover, the majority of weight lost in people randomized to TRE came from loss of lean mass rather than fat mass. The mechanism(s) underlying this phenomenon is unknown, and future studies will be aimed at exploring the relationship between fasting and lean body mass.

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List of Abbreviations

ALK: alkaline phosphatase

ALMI: Appendicular Lean Mass Index

ALT: alanine aminotransferase

AMY: Amylase

AST: Aspartate Transaminase

BHBA: beta-hydroxybutyric acid

BILT: Bilirubin Total

BMC: Bone Mineral Content

BMI: Body Mass Index

BUN: Blood Urea Nitrogen

Ca: Calcium

CI: Confidence Interval

Cl: Chloride

Cm: Centimeter

CMT: Consistent Meal Timing

CO2: Carbon Dioxide

CPK: Creatine Phosphokinase

CRE: Creatinine

CVD: Cardiovascular Disease

DLW: Doubly-labelled Water

DXA: Dual-energy X-ray absorptiometry

eTRE: early Time-restricted Eating

Fe: Iron

FFM: Fat-free Mass

FM: Fat Mass

G: gram

GGT: gamma-glutamyl transferase

Hb: Hemoglobin

HbA1c: Hemoglobin A1c

HC: Hip Circumference

HFD: High-fat Diet

IF: Intermittent Fasting

K: Potassium

Kcal: kilocalorie

Kg/m2: Kilogram per meter squared

Kg: Kilogram

LDH: Lactate Dehydrogenase

LDL: Low-density lipoprotein

LMM: Linear Mixed Model

Mg: Magnesium

MPS: Muscle Protein Synthesis

mRNA: messenger Ribonucleic Acid

Na: Sodium

NHANES: National Health and Nutrition Examination Survey

PBRC: Pennington Biomedical Research Center

PBRC: Pennington Biomedical Research Center

Phos: Phosphate

PSQI: Pittsburg Sleep Quality Index

RED Scale: Rewards-based Eating Drive Scale

RMR: Resting Metabolic Rate

RQ: Respiratory Quotient

SD: Standard Deviation

T2DM: Type 2 Diabetes Mellitus

TEE: Total Energy Expenditure

TP: Total Protein

TRE: Time-restricted Eating

TRF: Time-restricted Feeding

UCP: Uncoupling Protein

UCSF: University of California, San Francisco

UHCC: University of Hawaii Cancer Center

VCO2: Volume Carbon Dioxide

VO2: Volume Oxygen

WC: Waist Circumference

Chapter 1- INTRODUCTION

1.1 Obesity as a Public Health Concern

The prevalence of Americans classified as overweight (BMI between 25 to 30 kg/m2) or obese (BMI greater than 30kg/m2) has increased dramatically over recent decades. According to the Centers for Disease Control and Prevention, 42.4% of Americans are obese, up from 30.5% in 2000 (1). Individuals who are overweight and obese have a higher risk of type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), cancer and other chronic diseases (2). People with overweight and obesity often have worsened CVD risk markers including excess abdominal adiposity, elevated blood pressure, insulin resistance, atherogenic dyslipidemia, and increased systemic inflammation, and studies have shown that even modest weight reduction from diet and exercise can improve CVD risk (3). However, long-term adherence to lifestyle changes is difficult. Therefore, it is important to find novel lifestyle-modification interventions that are 1) effective in reducing weight and preventing weight gain and 2) accessible and straightforward to patients who struggle to maintain healthy habits.

1.2 Intermittent Fasting

Intermittent fasting (IF) has gained attention in recent years in scientific and popular literature as a method for weight loss. IF is an umbrella term that refers to a diet plan with eating windows separated by defined periods of fasting. The duration and frequency of fasting varies greatly between different intermittent fasting regimens, which makes it difficult to compare and contrast study results. There are many claims about the benefits of IF; however, most of these claims are either untested/undertested in humans or not unique to IF compared to traditional calorie restriction diets (4).

1.3 Time-Restricted Eating

Time-restricted eating (TRE) is a specific IF protocol that involves consistent fasting and eating periods that repeat on a 24-hour cycle. TRE has gained popularity as a weight loss method due to its inherent simplicity. TRE does not require tedious, unsustainable, and time-consuming methods such as calorie-counting nor does it require any special instructions or adherence to restrictive diets. These features make TRE an attractive weight loss strategy for those who struggle with excess weight issues.

1.4 Animal Models of Time-Restricted Eating

Time restricted feeding (TRF) prevents weight gain in mice challenged with a high-fat diet (HFD) (5). Importantly, mice fed under a TRF protocol (8-hour feed, 16-hour fast) ate the same quantity of calories per day as mice fed *ad libitum* (5). Whereas mice fed *ad libitum* eat small amounts of food consistently throughout the 24-hour cycle, TRF leads to a compensatory spike in food intake during the feeding window that offsets the calorie deficit imposed by fasting. Nonetheless, TRF resulted in significantly less weight gain despite similar caloric intake (5). Weight loss without a concomitant decrease in calorie intake suggests that TRF may affect resting metabolic rate (RMR) or total energy expenditure (TEE) to achieve a negative calorie balance.

The mechanism(s) underlying how TRF affects energy balance is unclear. Hatori et al. demonstrated that activity and energy expenditure increased slightly in TRF mice near the end of the nocturnal cycle (mice are nocturnal, so this is during normal waking hours) (5). Additionally, TRF induced rhythmic changes in hepatic fatty acid metabolism and bile acid production (5). Excess bile acid production can lead to elevated circulating bile acids, which leads to the induction of adaptive thermogenesis in brown adipose tissue (6). UCP mRNA expression in brown adipose

tissue of TRF mice demonstrated rhythmic increases that paralleled the increase in nocturnal energy expenditure (5). Therefore, it is possible that TRF may increase energy expenditure in mice through increased activity and increased adaptive thermogenesis. However, in humans brown adipose tissue accounts for a small percentage of total energy expenditure that is unlikely to influence weight loss(7). Therefore, further studies are required to determine if and how TRF leads to weight loss in humans.

A follow up study examined how TRF affects weight dynamics in already obese mice (8). Obese mice experienced a rapid weight reduction when switched from an *ad libitum* HFD to a TRF HFD (8). The reduction in weight was accompanied by a concomitant decrease in circulating leptin and hepatic triglyceride levels as well as improvements in fasted and fed insulin levels and glycemic control (8). These data suggest that TRF can provide both preventative and therapeutic effects (8).

1.5 Human Studies of Time-Restricted Eating

Most humans eat throughout the majority of their waking hours. A study of 156 subjects found that the median daily eating duration was nearly 15 hours per day, and less than 10% of subjects had an eating window less than 12 hours per day (9).

To date, only a few studies have examined the effects of TRE in adults who are overweight and obese. These studies are largely uncontrolled and small but demonstrate that TRE leads to reduced caloric intake and is associated with a decrease in body weight and/or fat mass (10-13).

One study examining the effects of a ten-hour eating window in patients with metabolic syndrome indicated that TRE reduces weight, blood pressure, and atherogenic lipids (13). Subjects' self-reported calorie intakes decreased by 8.62% relative to baseline, and subjects lost an average of 3.30kg (~3%) bodyweight. Importantly this was a single-arm study with no control intervention

and had 19 subjects total. Therefore, it is unknown whether the improvements in glucose homeostasis and CVD risk are unique to TRE or, rather, associated with general weight loss. Furthermore, it is unclear how much of the weight loss in this study can be attributed directly to TRE rather than the interventions associated with participation in a clinical trial.

In another study, 15 men were enrolled to a study examining the effects of TRE on glucose homeostasis and CVD risk (10). In this randomized crossover trial, subjects ate within an early (8am to 5pm) and late (12pm to 9pm) 9-hour eating window for 7 days. While TRE had no effects on fasting insulin, non-esterified fatty acids, or gastrointestinal hormones, TRE resulted in a significant improvement in glycemic control in response to a test meal. There was no effect of meal timing (early versus late) on glycemic control. However, the authors did observe a small but significant decrease in fasting glucose only in the eTRE group. While this study was relatively short and not intended as a weight loss trial, the authors observed a ~1% decrease in weight during the TRE regimens. Importantly, the subjects experienced similar weight loss during the baseline period in which subjects were instructed to consume their habitual diets. This suggests that diet monitoring in a clinical trial may be responsible for weight loss rather than the TRE regimen itself (10).

In another small study of men with prediabetes, TRE was shown to reduce signs of insulin resistance even in the absence of reduced energy intake (14). The isocaloric nature of this study provides compelling evidence that TRE offers unique benefits independent of weight loss.

To date, only one randomized controlled trial (RCT) has been published examining the effects of TRE in overweight and obese subjects (15). Subjects' daily eating window and number of eating occasions decreased in the TRE group relative to control; however, caloric intake was not measured in this study. This RCT, while small (n=20), demonstrated that TRE leads to significant

weight loss that is accompanied by a significant loss in total lean mass and visceral fat (15). However, glucose homeostasis and CVD risk markers did not significantly differ between TRE and control groups.

In summary, the current data from human trials suggests that TRE may improve glucose homeostasis independently of weight loss, and it is possible that eating window timing may contribute to this effect (10, 14). Additionally, one small RCT suggests that *ad libitum* TRE can lead to weight loss in overweight and obese subjects (15). However, current data suggests that TRE is not beneficial for weight loss when diets are isocaloric (14). Larger and better controlled RCTs are needed to determine whether *ad libitum* eating during a TRE protocol will lead to weight loss and whether *ad libitum* TRE will lead to improvements in glucose homeostasis or CVD risk markers.

1.6 Ramadan Fasting

Ramadan is a religious holiday that involves daily fasting from sunrise to sunset for one month. Due to the regular interval of periodic fasting, studies on Ramadan fasting can provide insights to the effects of TRE. Many studies have demonstrated that Ramadan fasting results in weight loss (16-20). Ramadan fasting is not associated with changes in RMR or TEE (19); rather, weight loss is associated with reduced caloric intake (17). Interestingly, one study found that Ramadan fasting resulted in increased caloric intake and slowed weight gain in subjects previously undergoing caloric restriction (21). This data suggests that fasting regimens may not be superior to other weight loss protocols for some individuals.

1.7 Adverse Effects of Time-Restricted Eating

There are rare reports of adverse effects associated with TRE including vomiting, headaches, increased thirst, constipation and diarrhea (14, 22). Studies on fasting during Ramadan have demonstrated that daily protein intake decreases (17, 18, 23) and some studies have shown a significant decrease in lean body mass (16, 18, 20). Further studies are warranted to determine TRE is associated with similar changes in protein intake and lean mass loss observed during Ramadan fasting. Caution should be taken when prescribing TRE to susceptible populations until more is known about TRE's effects on diet and body composition.

1.8 Hypothesis

Based on the current evidence surrounding TRE in rodents and humans, we hypothesized that an 8-hour TRE interval in individuals with overweight and obesity would lead to weight loss and favorable changes in metabolic markers compared to individuals following a standard 3-meals-per-day diet (Consistent meal timing- CMT). Since TRF was associated with decreased weight gain despite isocaloric food intake in rodents, we also hypothesized that TRE would increase RMR and/or TEE. To our knowledge, this is the first large and prospective randomized controlled trial designed to determine the effect of TRE on weight and comprehensive metabolic outcomes in overweight and obese subjects.

Chapter 2- EXPERIMENTAL MODEL AND SUBJECT DETAILS

2.1 Participant Recruitment and Eligibility

This study was conducted with approvals from the Institutional Review Board at the University of California, San Francisco (UCSF) and the University of Hawai'i Cancer Center (UHCC). Clinical trial registered as "Study of Time-restricted Eating on Weight Loss. A Randomized Controlled Trial of the Effects of Time-restricted Eating on Weight Loss in Obese Subjects." ClinicalTrials.gov NCT03393195.

Participants were recruited through targeted email campaigns to join a weight loss study investigating the effects of meal timing on weight loss using the Health eHeart research database, physical fliers posted at the UCSF campus, and Facebook advertisements. Subject eligibility was determined using an online eligibility survey using the inclusion and exclusion criteria listed in Table 2.1, and all eligible subjects completed an online consent form via the Eureka Research Platform.

An initial power analysis showed that with a standard deviation (SD) of 9 kg in measured weight, and conservatively assuming a intraclass correlation (ICC) of 0.8 between the baseline and follow-up weights, the clinical sample of 50 participants would provide 80% power in 2-sided 5% tests to detect a between-group difference in weight change of 5.0 kg.

In addition, before the data analysis was undertaken, we estimated the minimum detectable effects in the planned analyses using linear mixed models (LMMs) for daily weights measured at home by the virtual participants, without using the data. Specifically, we estimated that with gradual attrition of 20% of the sample by the end of the trial, 81 of 90 expected daily measurements per patient would on average be available. Then under the original assumption of an SD of 9 kg, and

considering ICCs between 0.8 and 0.95, we estimated that a sample of 100 virtual participants would provide 80% power in 2-sided 5% tests to detect between-group differences in weight change of .44 to .89 kg; in the observed sample of 116, corresponding estimates are 0.41 to 0.82 kg. In the clinical data, with single pre- and post-measurements, corresponding estimates under the same assumptions were 2.5 to 5.0 kg.

2.2 Study Activities and Treatment Groups

141 participants were enrolled in the study and data was collected from 116 subjects. 105 participants completed the 12-week protocol. The study was conducted on a custom mobile study app on the Eureka Research Platform. Participants received study surveys through the study app. Participants were given a Bluetooth weight scale to use daily, which was connected through the study app. Subjects were randomized to one of two eating plans. The study intervention only included recommendations to the timing of food intake and received daily reminders about their eating windows through the study app. In order to reduce bias between treatment groups, both study groups had similar levels of engagement with the study app and receive similar daily reminders about their eating schedules.

Treatment Groups:

1) The CMT group was instructed to eat three structured meals per day. Snacking between meals was permitted so long as it did not interfere with the participants ability to consume structured meals.
2) The TRE group was instructed to eat *ad libitum* from 12:00 pm until 8:00 pm and completely abstain from caloric intake from 8:00 pm until 12:00 pm the following day

(16h fast:8h eat). Only black coffee or tea and water were permitted outside of the eating widow.

Participants received a $50 Visa gift card for participating in the study. Subjects were not instructed to make any changes to their physical activity. Additionally, subjects were not instructed to make any changes to the quality, quantity, or caloric content of their diet in any way.

2.3 Randomization

Participants were self-enrolled upon completion of eligibility survey if all inclusion criteria were met. Interventions were assigned programmatically using the Ruby program. The Ruby program was written to create a schedule of strata and blocks based on gender, age, and BMI. The shuffle function was used to make blocks random. The schedule was filtered according to stratifiers from the eligibility survey and the next open slot in the schedule was assigned to a participant. Block randomization was used with random block sizes of 2 and 4 with equal distribution between study groups. Randomization schedule was generated on the backend servers of Eureka; the schedule was only visible to the programmer staff, and the sequence was concealed form the clinical staff.

2.4 Data Collection

2.4.1 Weight Measurements

All participants received an iHealth Lite Bluetooth weight scale (Model HS4S) to use at home. Participant accounts were linked to the Eureka Research platform so that data could be collected by the research team. Participants were instructed to use the scale daily in the morning before eating or drinking and prior to structured physical activity.

2.4.2 Estimated Energy Intake and Energy Expenditure

At-home weight measurements from the total cohort were used in a linear mathematical model of body weight dynamics to estimate energy intake and energy expenditure as described by Guo et al. (24). A 20-day interval was used to reduce variability in the model's estimated means of energy intake and energy expenditure.

2.4.3 Blood Pressure Measurements

A subset of the total cohort received a MOCACARE MOCACuff blood pressure cuff (Item model number 5031101001) to use at home. Participants were instructed to use the blood pressure cuff daily in the morning before eating or drinking and prior to structured physical activity. Participants were instructed to email their data to the study team upon completion of the study.

2.4.4 Sleep Analysis

Participants' sleep data was analyzed through the self-reported Pittsburgh Sleep Quality Index (PSQI). PSQI surveys were sent to the participants through the study app.

2.4.5 Food Attitudes Analysis

Participants received the Reward-Based Eating Drive Scale (RED Scale) surveys through the study app. The RED Scale survey is a validated, 9 item Likert scale survey to measure reward-based eating drive (25). RED Scale surveys were sent to the participants through the study app.

2.5 In-person Metabolic Testing

Participants who lived within 60 miles of UCSF were eligible to undergo extensive in-person metabolic testing at the UCSF Clinical Research Center and the UCSF Body Composition Laboratory if they had no barriers to performing the tests (able to stand unassisted for several

minutes, able to lie down for 30 minutes, no internal metal artifacts). In-person study activities were partly funded through the Shape Up! Adults study at the UHCC (NCT03637855). Participants who opted into the in-person testing group completed measurements detailed by Ng et al. (26). 50 participants opted into the in-person testing, and 46 participants completed all four in-person visits.

All four study visits began between 8:00-10:30 am, and subjects were instructed to fast starting at 8:00 pm the night before the study visit (fasting duration was matched between groups). The first study visit occurred ~1 week before the start of the participant's eating plan. During this visit, participants began their doubly-labelled water (DLW) total energy expenditure (TEE) measurement. ~1 week later at visit 2, participants came into the clinic fasted and provided 2 more urine samples to complete their baseline DLW TEE measurement. Additionally, participants provided blood samples, underwent resting metabolic rate measurements, Dual-energy X-ray absorptiometry (DXA) scans, manual anthropometrics, and muscle function testing. The participant's eating plan began the day after visit 2. Visit 3 occurred ~1 week prior to the end of the study. During visit 3, participants began their follow-up DLW TEE measurement. Visit 4 occurred on the last day of the study. At visit 4, participants completed their follow-up DLW TEE measurement, provided blood samples, underwent resting metabolic rate measurements, DXA scans, manual anthropometrics, and muscle function testing. Participants were compensated with a $50 Visa gift card for each completed study visit.

2.5.1 Blood Measurements

On visit 2 and visit 4, whole blood fasting sample of 40 ml was collected from each participant via venipuncture. All participants began fasting at 8:00pm the night before their blood draw, so duration of fasting for blood measures was matched between groups. Blood samples were placed

on ice and processed within 4 hours into plasma, serum, whole blood, and buffy coat components and stored at -80°C at each study site until analysis. Biochemical analyses of all lipid and blood chemistry profiles were performed at Pennington Biomedical Research Center (PBRC). Serum chemistry panels were assayed through the use of a DXC600 instrument (Beckman Coulter, Inc.; Brea, CA). Insulin was measured by immunoassay on an Immulite 2000 platform (Siemens Corporation; Washington, DC). Additionally, EDTA plasma was used for targeted metabolomic analysis.

2.5.2 Total Energy Expenditure Measurements

Total energy expenditure (TEE) analysis measured TEE for approximately 7 days prior to the study start and the last ~7 days of the study. Subjects reported to the clinic after an overnight fast. A pre-dose urine specimen was collected and transfer to a 5 mL tube with an elastic o-ring seal. Subjects consumed a weighed dose of doubly labeled water containing, on average, 1.8 g/kg and 0.12 g/kg total body water of 10AP 18O water and 99.9 AP 2H water, respectively (27). The container was washed with 50 mL and subjects drank that as well. Subjects voided at 2 hours after the dose and that specimen was discarded. Two additional specimens were collected at 3 and 4 hours after the dose, and aliquots were transferred to separate 5 mL o-ring sealed tubes. Urine specimens were stored frozen at -20ºC then sent in Styrofoam boxes cooled with frozen gel packets to the University of Wisconsin. Subjects fasted throughout the entire study protocol and were provided 250 mL water between dosing and three hours post-dose. The volumes of water between the dose and 3 hours were recorded and subtracted from the total body water. Subjects returned to the clinic ~7 days later after an overnight fast. Urine was collected at the beginning and end of the study visit (~2 hours apart) and aliquots were transferred to separate 5mL o-ring sealed tubes. Subjects fasted and abstained from fluid intake during the duration of this study visit. Follow-up

TEE measurements were collected during the last ~7 days of the study using the same protocol described above.

Specimens were refrozen until analyzed. As detailed by Thorsen et al (28), specimens were thawed and decolorized with 200 mg of dry carbon black. Specimens were passed through a syringe mounted 45-micron filter to remove carbon black and any other solids. Aliquots of 1 mL each were placed in an autoinjector vial and a 15mL septum topped tube for isotopic analysis. The 15mL tube was flushed with 0.4% CO2 in helium and 18O isotopic analyses performed on a Delta V Isotope Ratio Mass Spectrometer equipped with a Gas Bench inlet (ThermoFisher). The other aliquot was analyzed for 2H on a Delta Plus equipped with a HD Device using chromium reduction (ThermoFisher). Calibrated natural abundance and enriched working standards were analyzed along with each batch and results expressed on the SMOW scale. Precisions for 18O and 2H were 0.15 permil and 1.0 permil, respectively.

The rate of CO2 production was calculated using equation A6 (29) as modified by Racette et al. (30). Total daily energy was calculated from rCO2 assuming a respiratory ratio of 0.86 using the Weir equation (31). Precision is 5% (27).

2.5.3 Resting Metabolic Rate and Respiratory Quotient Measurements

Resting metabolic rate (RMR) measurements and respiratory quotient (RQ) measurements were collected using the PARVO Medics TrueOne 2400 Metabolic Cart (PARVO Medics, Salt Lake City, UT, USA) using the manufacturer's instructions. Briefly, fasted subjects rest for 10 minutes prior to start of experiment. A canopy is placed over participant's head and dilution pump flow rate is adjusted so that CO2 dilution is between 1-2%. Measurements are recorded for 30 minutes. The first 5 minutes of measurements were omitted. Once steady state was achieved (coefficient

of variance <10% for VO2 and VCO2), 5 minutes of data was collected and averaged to produce RQ and RMR values.

2.5.4 Dual-Energy X-Ray Absorptiometry Analysis

Dual-Energy X-Ray Absorptiometry (DXA) measures were collected using an adaptation from the protocol described by Ng at al. (32). In the current study, two whole-body DXA scans were performed with repositioning using a Hologic Horizon/A system (Hologic Inc., Marlborough, MA, USA) and the results averaged. Participants were scanned according to the manufacturer's guidelines. All DXA scans were analyzed at UHCC by a single certified technologist using Hologic Apex 5.5 software. Lean mass was calculated by subtracting bone mineral content (BMC) from fat-free mass (FFM). Appendicular lean mass was calculated by adding the lean mass of both arms and legs. Trunk lean mass was calculated by subtracting the BMC of the spine, pelvis, and ribs from trunk FFM.

2.5.5 Manual Anthropometrics

Anthropometric measures of waist circumference (WC) and hip circumference (HC) were collected using a flexible measuring tape according to the standard protocol from National Health and Nutrition Examination Survey (NHANES) (Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey (NHANES): Anthropometry Procedures Manual, 2007. Version current 30 October 2018. Internet: https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/manuals.aspx?BeginYear=2017 (accessed 18 August 2019).). Measurements were recorded in triplicate to the nearest 0.1 cm and results averaged. If a measurement differed by greater than 1 cm, a fourth measurement was taken and the closest three measurements averaged.

2.5.6 Muscle Function Testing

Muscle function testing was adapted from the protocol described by Ng et al (22). Isokinetic and isometric right leg strength were measured using a Biodex System 4 (Biodex Medical Systems Inc) dynamometer. For isometric measurements, the dynamometer was fixed at 60° from full extension. For the isokinetic measurement, resistance was set at 60°/s. Peak torque was recorded as the maximum torque achieved during the repetitions. Hand grip strength for the right and left arms was measured with a handgrip dynamometer (JAMAR 5030J1). Participants positioned their elbow at a 90° angle and were asked to squeeze the dynamometer as hard as they could. The strength of each hand was measured in kilograms, and the average of the 3 measurements was recorded.

2.5.7 Oura Ring Analysis

Only participants who used an iPhone were eligible to receive an Oura ring (Oura, Oulu, Finland). Participants were fitted with an Oura ring to wear on any finger. Participants were instructed to wear the ring during day and night and remove the ring only for charging and activities that would require removal of the ring.

2.6 Statistical Analysis

The primary outcome was change in weight since baseline, measured daily via iHealth, in the overall cohort of 100 participants. To estimate the intention-to-treat effect of treatment assignment, we used a linear mixed model (LMM) with fixed effects for treatment assignment, days since baseline, and their interaction, and random effects for participant and day, with unstructured covariance matrix, accommodating any non-linearity in the trajectories using 3-knot cubic splines. The treatment effect was estimated by the fitted between-group difference at day 90, net of any

baseline difference. In sensitivity analyses, repeated the analysis after Winsorizing outliers, which was defined as points more than 1.5 times the interquartile range below the 25th or above the 75th percentile of the overall distribution. No adjustments were made to p-values or confidence intervals for multiple comparisons for the primary outcome.

Our secondary outcomes included differences in weight loss, body fat, lean mass, fasting glucose, insulin, and Hemoglobin A1c (HbA1c) levels, resting metabolic rate, and total energy expenditure, assessed at the baseline and 12-week clinical visits for a subset of 46 participants. To estimate the intention to treat effect of treatment assignment on changes in these outcomes, we used LMMs with fixed effects for treatment assignment, an indicator for the 12-week visit, and their interaction, and a random effect for participant. The treatment effect was estimated by the interaction. In sensitivity analyses, we repeated these analyses Winsorizing any outliers, defined as in the primary analysis. P-values and confidence intervals will be Bonferroni-corrected for 8 comparisons.

All other outcomes measured at the baseline and 12-week clinical visits were considered exploratory and analyzed using the methods described for the secondary outcomes, without penalization for multiple comparisons. Data are presented as mean (95% confidence intervals) unless otherwise noted.

2.7 Table of Inclusion and Exclusion Criteria

Table 2.1. Inclusion and exclusion criteria. Below are the inclusion and exclusion criteria used to determine subject eligibility. Note: The original inclusion criteria was BMI between 30 and 40 kg/m2 and also excluded subjects who have tried more than 1 structured diet within 6 months of recruitment. BMI requirement was expanded and structured diet limit was dropped to increase study enrollment.

Inclusion Criteria (n excluded)	**Exclusion Criteria (n excluded)**
Male or female ages 18-64 (n=5 >64)	Current or past cancer diagnosis (n=21)
BMI between 27kg/m^2 and 43 kg/m^2 (<27 n=348, >43 n=72)	Pregnancy, breastfeeding, or planned pregnancy within 6 months (n=21)
Regularly consume breakfast (≥5 days/week) (n=566)	Current diagnosis of type 1 or type 2 Diabetes Mellitus (n=177)
Willing and able to skip breakfast (n=761)	Currently taking glucose-lowering drugs (n=133) or weight loss pills (n=116)
Speak, read, comprehend English	History of gastric bypass surgery or any weight loss surgery (n=66)
Access to reliable internet and/or Wi-Fi	>15% weight fluctuation in past 5 years (n=467)
Have valid email address and phone number	History of anorexia or bulimia (n=39)
	Frequent travel across time zones (n=99) or unusual work hours (n=182)
	Unable to fast for prolonged periods (n=168)

Chapter 3- RESULTS

3.1 Study Participant Baseline Characteristics

Participants were recruited between August 2018 and June 2019 and data collection was completed in October 2019. Data were collected and analyzed for all 116 subjects who participated in the study; 105 out of 116 (90.5%) completed the 12-week intervention (Figure 3.1). Of the 36 randomized participants who did not complete the study, 25 never recorded weight measurements (TRE n= 10, CMT n=15), 8 were lost to follow-up (TRE n=7, CMT n=1), and 3 discontinued intervention (TRE n=2, CMT n=1). Participants had a mean (SD) age of 46.5 (10.5) years and a mean (SD) weight of 99.2 (16.0) kg (Table 3.1). At baseline (0 weeks), there were no differences between groups in weight or age. Self-reported adherence to the diets was 92.34% in the CMT group (did not miss any meals) and 83.48% in the TRE group (ate only within their 8-hour feeding window) (Figure 3.2A).

Of the 141 participants recruited to the study, 50 participants opted to undergo comprehensive in-person metabolic testing (referred to as in-person cohort). 46 out of 50 participants completed the in-person testing (CMT n=24, TRE n=22). Baseline characteristics of the in-person cohort are shown in Table 3.2.

3.2 Change in Weight

In the total cohort, there was a significant decrease in weight in the TRE group (-0.94kg, 95% CI, -1.68, 0.20, p=0.013) and a non-significant decrease in weight in the CMT group (-0.68kg, 95% CI, -1.41, 0.05, p=0.07). Importantly, there was no significant difference in weight change between groups (-0.26kg, 95% CI -1.30, 0.78, p=0.63) (Figure 3.2B,C, Table 3.3A). There was a significant decrease in percentage of baseline weight in the TRE group (-1.17%, 95% CI, -1.89, -0.45,

p=0.002) and in the CMT group (-0.75%, 95% CI, -1.47, -0.037, p=0.039); however, there was no significant difference between groups (-0.41%, 95% CI, -1.43, 0.60, p=0.43) (Table 3.3A). There were no statistically significant changes in estimated energy intake or energy expenditure between groups (Figure 3.3A and 3.3B).

Within the in-person cohort (n=50), there was a significant decrease in weight in the TRE group using the in-person weight measurements (-1.70kg, 95% CI -2.56, -0.83, p<0.001) but not in the CMT group (-0.57kg, 95% CI, -1.40, 0.26, p=0.18) (Table 3.3B, Figure 3.2D). There was a non-significant difference in weight loss between groups (-1.13kg, 99.7% CI, -2.33, 0.07, p=0.07) (Table 3.3B). There was a significant decrease in percentage of baseline weight in the TRE group (-1.81%, 95% CI, -2.85, 0.78, p<0.001) but not in the CMT group(-0.65%, 95% CI, -1.64, 0.34, p=0.19) or between groups (-1.16%, 95% CI, -2.59, 0.27, p=0.11). There was strong agreement between in-person weight measurements and at-home weight measurements as determined by a Bland-Altman analysis (Figure 3.4).

3.3 Body Composition Changes

We measured body composition and energy expenditure in the in-person cohort (n=46). As measured by DXA, there was no significant change in whole body fat mass (FM) in the TRE (-0.51kg, 95% CI, -1.17, 0.15, p=0.13) or the CMT groups (-0.03kg, 95% CI, -0.66, 0.60, p=0.93), and there was no significant difference between groups (-0.48kg, 99.7% CI, -1.75, 0.79, p=0.30) (Table 3.3B). There was a significant decrease in lean mass (calculated as fat-free mass minus bone mineral content) in the TRE (-1.10kg, 95% CI, -1.73, -0.48, p<0.001) but not in the CMT group (-0.35kg, 95% CI -0.95, 0.25, p=0.25). There was no significant difference in lean mass between groups (-0.75kg, 99.7% CI, -1.96, 0.45, p=0.09). Appendicular lean mass (ALM) was decreased significantly in the TRE group (-0.64kg, 95% CI, -0.89, -0.39, p<0.001) but not in the

CMT group (-0.17kg, 95% CI, -0.41, 0.07, p=0.16), and there was a significant difference between groups (-0.47kg, 95% CI, -0.82, -0.12, p=0.009). There was a significant decrease in appendicular lean mass index (ALMI) in the TRE group (-0.220kg/m2, 95% CI, -0.301, -0.139, p<0.001) but not in the CMT group (-0.058kg/m2, 95% CI, -0.136, 0.020, p=0.14). The difference in ALMI between groups was also significant (-0.162kg/m2, 95% CI, -0.274, -0.050, p=0.005). Trunk lean mass significantly decreased in the TRE group (-0.47kg, 95% CI, -0.88, -0.06, p=0.024). There was no significant change in trunk lean mass in the CMT group (-0.15kg, 95% CI, -0.54, 0.24, p=0.45) or between groups (-0.32kg, 95% CI, -0.89, 0.25, p=0.27).

3.4 Changes in Energy Expenditure

Respiratory quotient (RQ) did not change significantly in the TRE group (0.0028, 95% CI, -0.021, 0.027, p=0.82); however, RQ did increase significantly in the CMT group (0.0348, 95% CI, 0.0012, 0.058, p=0.003), but there was no significant difference between groups (-0.0320, 95% CI, -0.065, -0.0009, p=0.06). There was no significant difference in resting metabolic rate (RMR) in the TRE (-28.1kcal/day, 95% CI, -91.8, 35.5, p=0.39) or the CMT group (-43.15kcal/day, 95% CI, -104.2, 18.0, p=0.17), and there was no significant difference between groups (15.0kcal/day, 99.7% CI, -108.1, 138.0, p=0.74) (Table 3.3B). There was a significant decrease in total energy expenditure (TEE) in both groups (TRE: -177.9kcal/day, 95% CI, -285.9, -69.9, p=0.001; CMT: -127.3, 95% CI -230.7, -23.9, p=0.016). There was no significant difference in change of TEE between groups (-50.6kcal/day, 99.7% CI, -259.2, 158.1, p=0.51).

3.5 Insulin and Glucose Homeostasis

Blood was collected after an overnight fast. Fasting times were matched between groups. There was no significant difference in fasting insulin in the TRE group (effect size, -0.50mU/L, 95% CI,

-2.34, 1.35, p=0.60), in the CMT group (effect size, 0.19mU/L, 95% CI, -1.58, 1.96, p=0.83), or between groups (effect size, -1.35mU/L, 99.7% CI, -3.25, 1.86, p=0.60) (Table 3.4). Fasting glucose did not significantly change in the TRE (effect size, -1.06mg/dL, 95% CI, -3.87, 1.75, p=0.46) or the CMT group (effect size, 0.29mg/dL, 95% CI -2.41, 3.00, p=0.83) and did not significantly differ between groups (effect size -1.35mg/dL, 99.7% CI, -5.25, 2.54, p=0.52). There was no significant difference in HOMA-IR in the TRE (effect size, -0.160, 95% CI -0.590, 0.270, p=0.47), in the CMT group (effect size, 0.085, 95% CI, -0.328, 0.498, p=0.69), or between groups (effect size, -0.245, 95% CI, -0.841, 0.351, p=0.42). HbA1C levels did not significantly change in the TRE group (effect size, -0.024%, 95% CI, -0.077, 0.029, p=0.37), the CMT group (effect size, -0.006%, 95% CI, -0.057, 0.044, p=0.81), or between groups (effect size, -0.018%, 99.7% CI, -0.120, 0.084, p=0.63).

3.6 Blood lipids and Cardiovascular Markers

There were no significant within or between group differences in triglycerides, total cholesterol, low-density lipoprotein (LDL), or high-density lipoprotein (HDL) (Table 3.5). There was no significant difference in systolic blood pressure within the TRE group (effect size, -1.7mmHg, 95% CI, -5.5, 2.2, p=0.39), but there was a significant decrease in the CMT group (effect size, -3.9mmHg, 95% CI, -7.6, 0.1, p=0.042). There was no significant between group difference in systolic blood pressure (effect size, 2.2mmHg, 95% CI, -3.2, 7.5, p=0.43). There was a significant change in diastolic blood pressure within the TRE group (effect size, -4.1mmHg, 95% CI, -8.1, -0.1, p=0.047) but not in the CMT group (effects size, -3.0mmHg, 95% CI, -6.9, 0.9, p=0.13) or between groups (effect size, -1.1mmHg, 95% CI, -6.7, 4.5, p=0.71).

A subset of the total cohort participants received a MOCACARE blood pressure cuff to measure blood pressure daily at home (CMT n=23, TRE n=16). There was no significant change systolic

blood pressure in the TRE group (effect size, -2.5mmHg, 95% CI, -7.0, 2.0, p=0.28) (Table 3.6). There was a significant decrease in systolic blood pressure the CMT group (effect size, -5.4mmHg, 95% CI, -9.1, -1.6, p=0.005). There was no significant difference in systolic blood pressure between groups (effect size, 2.9mmHg, 95% CI, -3.0, 8.8, p=0.34). Diastolic blood pressure decreased in the TRE group (effect size, -3.6mmHg, 95% CI, -6.3, -0.8, p=0.011) but not in the CMT group (effect size, -2.1mmHg, 95% CI, -4.4, 0.2, p=0.07). There was no significant difference in diastolic blood pressure between groups (effect size, -1.5mmHg, 95% CI, -5.0, 2.1, p=0.42).

3.7 Other blood markers

There was a significant decrease in serum gamma-glutamyl transferase (GGT) in the TRE group (effect size -1.86U/L, 95% CI, -3.65, -0.07, p=0.042) and in the CMT group (effect size -2.22U/L, 95% CI, -3.93, -0.50, p=0.011), but the differences were not significant between groups (effect size, -0.36U/L, 95% CI, -2.12, 2.84, p=0.8) (Table 3.7). There was a significant decrease in serum alanine aminotransferase (ALT) in the TRE group (effect size -2.40U/L, 95% CI, -4.75, 0.06, p=0.045) and in the CMT group (effect size -3.38U/L, 95% CI, -5.63, -1.13, p=0.003), but the differences were not significant between groups (effect size, 0.98U/L, 95% CI, -2.27, 4.23, p=0.55). There was no significant change in uric acid levels in the TRE group, but there was a significant increase in uric acid in the CMT group (effect size, 0.401umol/L, 95% CI, 0.096, 0.706, p=0.01), and the difference between groups was also significant (effect size, -0.595 µmol/L, 95% CI, -1.034, -0.155, p=0.008). There was a significant decrease in lactate dehydrogenase (LDH) levels in the CMT group (effect size -9.92 U/L,95% CI, -19.80, -0.04, p=0.049) and this difference was significant between groups (effect size 14.37 U/L, 95% CI, 0.14, 28.60, p=0.048). There were no significant changes in any other serum markers (albumin, alkaline phosphatase (ALK), amylase

(AMY), aspartate transaminase (AST), beta-hydroxybutyric acid (BHBA), bilirubin total (BILT), blood urea nitrogen (BUN), calcium (Ca), chloride (Cl), carbon dioxide (CO2), creatine phosphokinase (CPK), creatinine (CRE), iron (Fe), Hemoglobin (Hb), potassium (K), magnesium (Mg), sodium (Na), phosphates (Phos), or total protein (TP)).

3.8 Subjective Sleep Quality and Food Attitudes

The total cohort received online surveys to measure subjective Rewards-Based Eating Drive (RED Scale) and sleep quality (PSQI) Using the self-reported RED Scale, we observed no significant changes in reward-based eating drive within groups or between groups (Table 3.8). We also did not observe any significant changes in overall sleep quality in either group or between groups in the total cohort (Table 3.8).

3.9 Oura Ring Analysis of Sleep and Activity Metrics

A subset of the in-person cohort (TRE n=17, CMT n=17) received an Oura ring to track sleep and activity. In this exploratory analysis, we observed a significant decrease in sleep efficiency (percentage of time asleep after going to bed) in the TRE group (effect size, -2.68%, 95% CI -4.82, -0.55, p=0.014) and between groups (effect size, -3.58%, 95% CI -6.62, -0.53, p=0.021), but no significant change in the CMT group (effect size, 0.89%, 95%CI -1.28, 3.06, p=0.42) (Table 3.9). There was a significant reduction in sleep efficiency score in the TRE group (effect size, -5.22, 95% CI, -9.50, -0.094, p=0.017) and between groups (effect size, -6.96, 95% CI, -13.06, -0.85, p=0.026) and no significant change in the CMT group (effect size, 1.74, 95% CI, -2.62, 6.09, p=0.43). There was also a significant reduction in sleep latency score in the TRE group (effect size -2.94, 95% CI, -5.57, -0.32, p=0.028) but not in the CMT group (effect size, 1.03, 95% CI, -1.69, 3.74, p=0.46), and the difference between groups was significant (effect size, -3.97, 95% CI, -7.75,

-0.19, p=0.039). There was a significant increase in awake time (time awake in bed) in the TRE group (effect size, 898.53seconds, 95% CI, 195.68, 1601.37, p=0.012) and between groups (effect size, 1313.79seconds, 95% CI, 311.32, 2316.26, p=0.011) but no significant change in the CMT group (effect size, -415.26seconds, 95% CI, -1130.07, 299.55, p=0.25). Together, these data suggest that TRE may lead to disrupted sleep and trouble falling asleep.

There was a significant reduction in daily movement in the TRE group (effect size, -2100 a.u., 95% CI, -3160, -1040, p<0.001) and between groups (effect size, -1670 a.u., 95% CI, -3210, -136, p=0.033) but not in the CMT group (effect size, -429 a.u., 95% CI, -1540, 685, p=0.45). There was a significant decrease in step count in the TRE group (effect size, -25.0 steps, 95% CI, -3940, -1060, p<0.001) but not in the CMT group (effect size, -257 steps, 95% CI, -1760, 1240, p=0.74), and there was a significant difference between groups (effect size, -2240 steps, 95% CI -4320, -162, p=0.035). The correlation between change in step count and change in TEE was 0.52 in the TRE group and 0.03 the CMT group, but the two correlations did not differ significantly (equality of correlations p=0.48) (Figure 3.5). Since TRE did not affect RMR (see Table 3.3), these data suggest that a reduction in physical activity may account for the observed decrease in TEE in the TRE group.

3.10 Figures and Tables

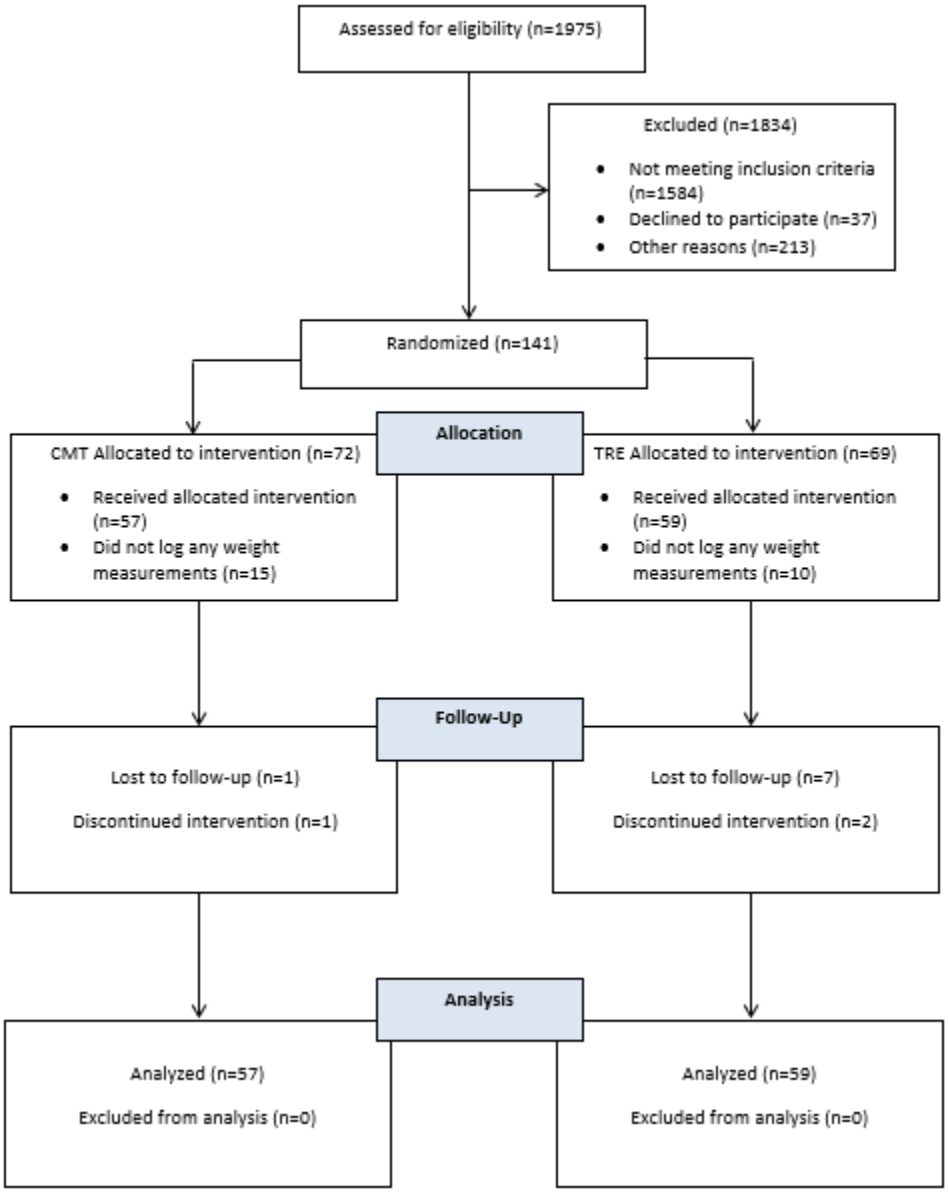


Figure 3.1. CONSORT Flow Diagram.

CONSORT flow diagram describing process of participant recruitment, enrollment, randomization, and data analysis. Subjects were excluded from participating if they 1) were older than 64 (n=5) 2) were <27kg/m^2 (n=348) or >43 kg/m^2 (n=72) 3) did not regularly consume breakfast (n=566) 4) were unwilling or unable to skip breakfast (n=761) 5) had a current or past cancer diagnosis (n=21) 6) were breastfeeding, pregnant, or planned to be pregnant within 6 months (n=21) 7) had current diagnosis of Type 1 or Type 2 Diabetes Mellitus (n=177) 8) were taking glucose lowering drugs (n=133) or weight loss pills (n=116) 9) had a history of gastric bypass or any weight loss surgery (n=66) 10) had a >15% weight fluctuation in past 5 years (n=467) 11) had a history of anorexia or bulimia (n=39) 12) frequently traveled across time

zones (n=99) or worked unusual work hours (n=182) 13) were unable to fast for prolonged periods (n=168). CMT, consistent meal timing group. TRE, time-restricted eating group.

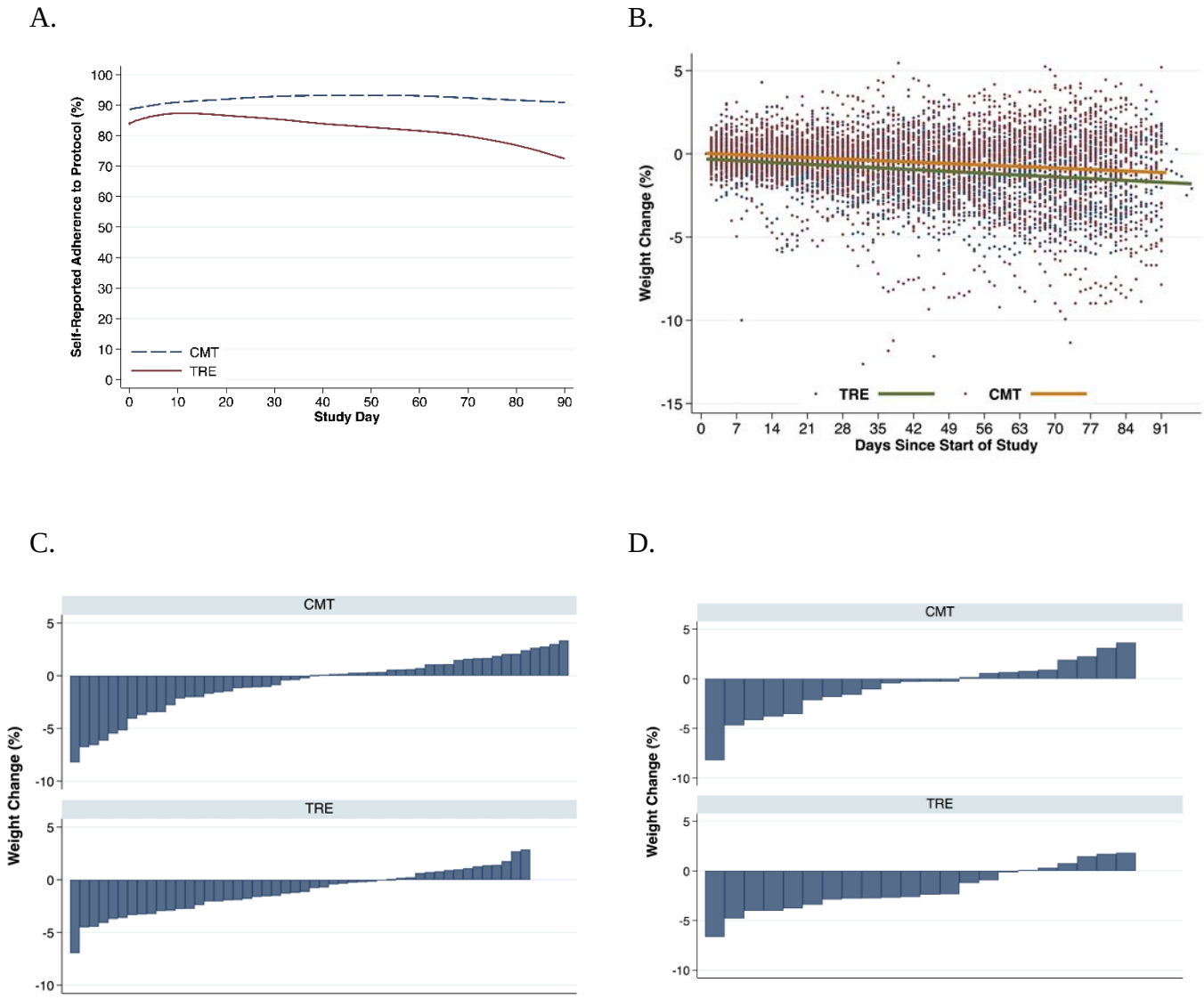
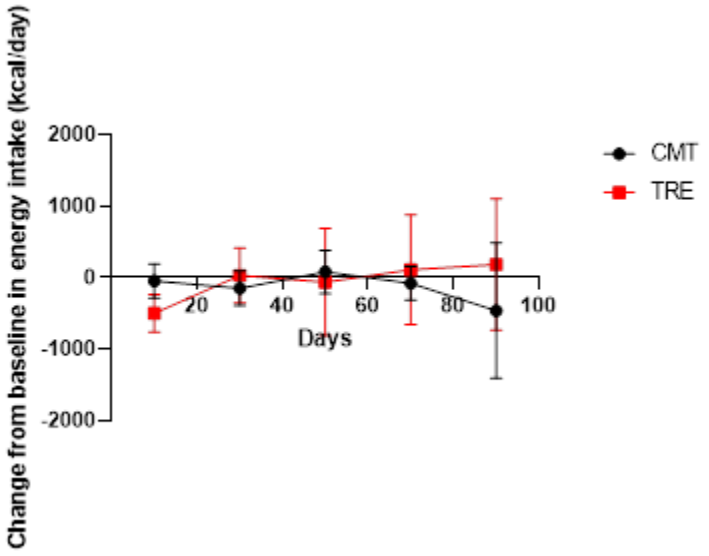


Figure 3.2. Adherence and weight change in total cohort and in-person cohort.

A. Participants were sent daily adherence surveys through the study app ("Did you adhere to your eating plan on the previous day?" Yes/No). Responses from all completed surveys were analyzed. A total of 1,058/2,160 responses were recorded from CMT participants (n=39) and 1,326/1,980 responses from TRE participants (n=41). **B.** Summary of total cohort daily weight measurements throughout the duration of the study in CMT (n=57) and TRE (n=59) groups. **C.** Waterfall plot showing percent weight change for each participant from the total cohort in the CMT group (top) and TRE group (bottom). **D.** Waterfall plot showing percent weight change for each in-person participants in the CMT group (top, n=24) and TRE group (bottom, n=22).

A.



B.

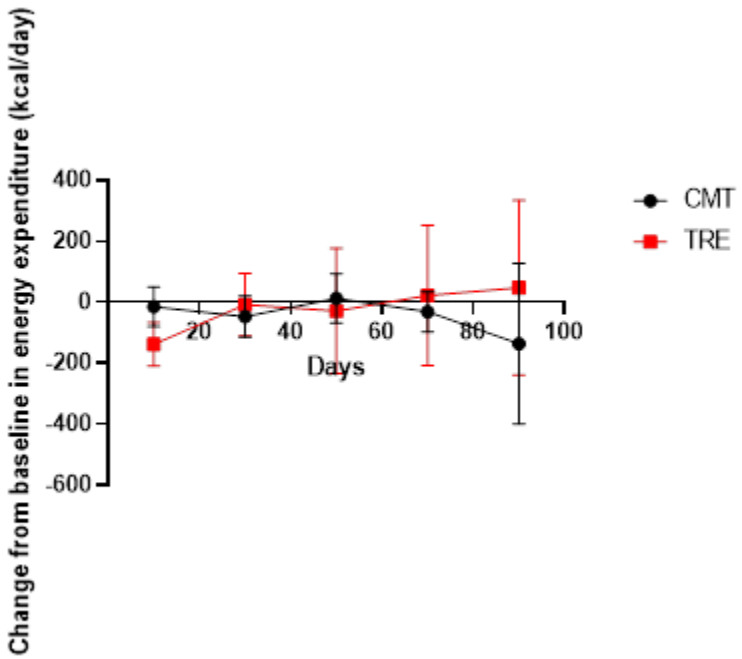


Figure 3.3. Estimated energy intake and energy expenditure.

Estimated changes from baseline in energy intake (A) and energy expenditure (B) derived from at-home weight measurements in total cohort.

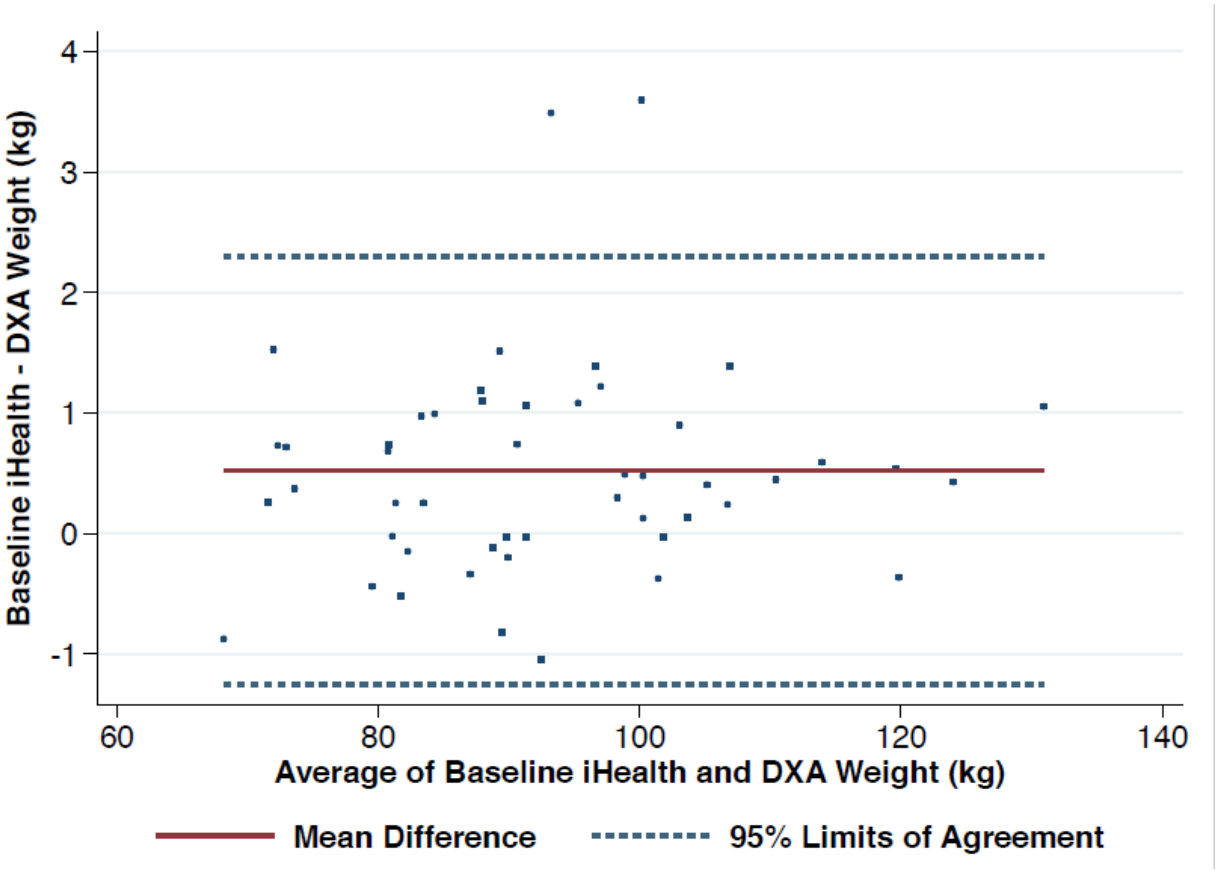


Figure 3.4. Bland-Altman plot.

Bland-Altman plot for in-person weights measured at the CTSI clinic and at-home weights using the iHealth Bluetooth scale.

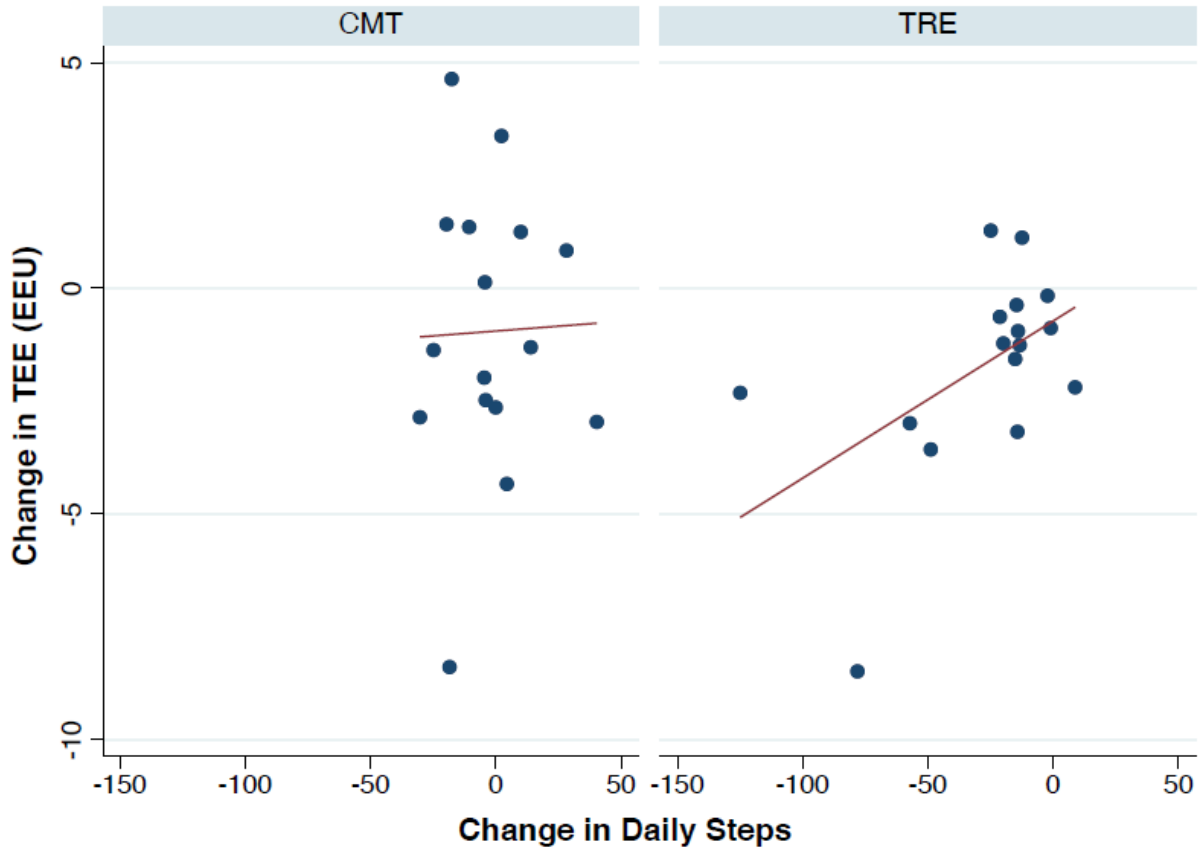


Figure 3.5. Correlation of change in step count and change in TEE.

The correlation of change in step count versus change in TEE was stronger in the TRE group (0.52) than the CMT group (0.03); however, the equality of correlations was not significant (p=0.48).

Table 3.1. Baseline Characteristics. Baseline characteristics for total cohort. Mean (SD) age weight, and BMI for all participants who participated in the study.

Total Cohort			
	Total (n=116)	CMT (n=57)	TRE (n=59)
Age	46.5 (10.5)	46.1 (10.3)	46.8 (10.8)
Female	46 (39.7%)	22 (38.6%)	24 (40.7%)
Male	70 (60.3%)	35 (61.4%)	35 (59.3%)
Weight (kg)	99.2 (16.0)	99.1 (15.1)	99.3 (16.9)
BMI (kg/m^2)	32.7 (4.2)	32.6 (3.4)	32.9 (4.9)

Table 3.2. In-Person Cohort Baseline Characteristics. Baseline characteristics for in-person cohort. Mean (SD) age for all in-person participants who participated in the study.

In-person Cohort			
	Total (n=50)	CMT (n=25)	TRE (n=25)
Age	43.8 (11.2)	44.4 (10.7)	43.3(11.8)
Female	22 (44.0%)	10 (40.0%)	12 (48.0%)
Male	28 (56.0%)	15 (60.0%)	13 (52.0%)
Black	2 (4.0%)	0 (0.0%)	2 (8.0%)
White	25 (50%)	16 (64.0%)	9 (36.0%)
Latinx	7 (14.0%)	3 (12.9%)	4 (16.0%)
Asian	12 (24.0%)	5 (20.0%)	7 (28.0%)
Other/Multi	4 (8.0%)	1 (4.0%)	3 (12.0%)
Weight (kg)	92.8 (14.2)	93.0 (13.3)	92.6 (15.2)
BMI (kg/m^2)	31.4 (4.0)	31.3 (3.5)	31.5 (4.5)

Table 3.3. Body composition and energy expenditure measurements from in-person cohort. All data is presented as means (95% confidence interval). For secondary outcome measures, Bonferroni-corrected confidence intervals are presented and Bonferroni-adjusted critical alpha of 0.006 is used. For data with statistical outliers, winsorised data was used to generate *p* values.

[&]secondary outcome using a Bonferroni-corrected alpha of 0.006 and presenting 99.7% CI for between group differences

*within-group *p* value less than 0.05

**within group *p* value less than 0.01

***within group *p* value less than 0.001

##between group *p* value less than 0.01

[δ]data analyzed only for completers. CMT n=24, TRE n=22

A.

	CMT Pre	CMT Post	ΔCMT	ΔCMT *p* value	TRE Pre	TRE Post	ΔTRE	ΔTRE *p* value	Difference between groups	*p* value
Total Cohort (iHealth weight measurements)	n=57 included in analysis				n=59 included in analysis					
iHealth Weight (kg)	99.2 (95.1, 103.3)	98.5 (94.3, 102.7)	-0.68 (-1.41, 0.05)	**0.07**	99.2 (95.1, 103.2)	98.2 (94.1, 102.4)	-0.94 (-1.68, -0.20)	**0.013***	-0.26 (-1.30, 0.78)	**0.63**
Weight Change (%)			-0.75 (-1.47, -0.037)	**0.039***			-1.17 (-1.89, -0.45)	**0.002****	-0.41 (-1.43, 0.60)	**0.43**

B.

In-person Cohort	CMT Pre (n=25)	CMT Post (n=24)	ΔCMT	ΔCMT *p* value	TRE Pre (n=25)	TRE Post (n=22)	ΔTRE	ΔTRE *p* value	Difference between groups	*p* value
Weight (kg) [&]	93.0 (87.4, 98.5)	92.4 (86.9, 97.9)	-0.57 (-1.40, 0.26)	**0.18**	92.6 (87.0, 98.1)	90.9 (85.3, 96.4)	-1.70 (-2.56, -0.83)	**<0.001*****	-1.13 (-2.33, 0.07)	**0.07**
Weight Change (%)			-0.65 (-1.64, 0.34)	**0.19**			-1.81 (-2.85, -0.78)	**<0.001*****	-1.16 (-2.59, 0.27)	**0.11**
Fat Mass (kg) [&]	30.7 (27.7, 33.7)	30.6 (27.6, 33.6)	-0.03 (-0.66, 0.60)	**0.93**	30.3 (27.3, 33.3)	29.8 (26.8, 32.8)	-0.51 (-1.17, 0.15)	**0.13**	-0.48 (-1.75, 0.79)	**0.3**

In-person Cohort	CMT Pre (n=25)	CMT Post (n=24)	ΔCMT	ΔCMT *p* value	TRE Pre (n=25)	TRE Post (n=22)	ΔTRE	ΔTRE *p* value	Difference between groups	*p* value
Percent Fat Mass (%)	33.0 (30.4, 35.7)	32.9 (30.3, 35.6)	-0.07 (-0.55, 0.42)	**0.78**	32.9 (30.3, 35.6)	32.8 (30.2, 35.5)	-0.09 (-0.59, 0.42)	**0.74**	-0.02 (-0.72, 0.68)	**0.96**
Visceral Fat mass (kg)	0.625 (0.529, 0.721)	0.634 (0.537, 0.730)	0.0088 (-0.0188, 0.0364)	**0.53**	0.579 (0.483, 0.675)	0.576 (0.480, 0.673)	-0.0026 (-0.0314, 0.0263)	**0.86**	-0.0114 (-0.0513, 0.0285)	**0.58**
Subcutaneous Fat Mass (kg)	1.95 (1.74, 2.17)	1.94 (1.72, 2.16)	-0.013 (-0.066, 0.040)	**0.63**	1.87 (1.66, 2.09)	1.84 (1.62, 2.06)	-0.038 (-0.093, 0.017)	**0.17**	-0.025 (-0.101, 0.051)	**0.51**
Lean mass (kg) [&]	59.7 (55.3, 64.1)	59.3 (55.0, 63.7)	-0.35 (-0.95, 0.25)	**0.25**	60.0 (55.6, 64.4)	58.9 (54.5, 63.3)	-1.10 (-1.73, -0.48)	**<0.001*** **	-0.75 (-1.96, 0.45)	**0.09**
Trunk lean mass (kg)	30.5 (28.3, 32.6)	30.3 (28.2, 32.5)	-0.15 (-0.54, 0.24)	**0.45**	30.4 (28.3, 32.6)	30.0 (27.8, 32.1)	-0.47 (-0.88, -0.06)	**0.024***	-0.32 (-0.89, 0.25)	**0.27**
Appendicular lean mass (kg)	25.8 (23.6, 28.0)	25.6 (23.4, 27.8)	-0.17 (-0.41, 0.07)	**0.16**	26.1 (24.0, 28.3)	25.5 (23.3, 27.7)	-0.64 (-0.89, -0.39)	**<0.001*** **	-0.47 (-0.82, -0.12)	**0.009[##]**
Appendicular Lean Mass Index (kg/m^2)	8.62 (8.10, 9.14)	8.56 (8.04, 9.08)	-0.058 (-0.136, 0.020)	**0.14**	8.80 (8.28, 9.32)	8.58 (8.06, 9.10)	-0.220 (-0.301, -0.139)	**<0.001*** **	-0.162 (-0.274, -0.050)	**0.005[##]**
Total Body Water (kg)[§]	42.7 (39.6, 45.8)	42.1 (39.0, 45.2)	-0.59 (-1.06, -0.13)	**0.013***	41.9 (38.6, 45.1)	41.5 (38.3, 44.7)	-0.36 (-0.85, 0.13)	**0.14**	0.23 (-0.44, 0.91)	**0.5**
Bone Mineral Content (g)	2541.9 (2388.3, 2695.5)	2546.9 (2393.3, 2700.5)	5.00 (-8.33, 18.33)	**0.46**	2511.3 (2357.7, 2664.9)	2523.2 (2369.6, 2676.9)	11.95 (-1.97, 25.87)	**0.09**	6.95 (-12.32, 26.23)	**0.48**
Waist Circumference (cm)	106.6 (102.3, 110.8)	105.9 (101.6, 110.2)	-0.69 (-4.28, 2.90)	**0.71**	106.3 (102.1, 110.5)	104.5 (100.1, 108.9)	-1.81 (-5.53, 1.92)	**0.34**	-1.12 (-6.29, 4.05)	**0.67**
Hip Circumference (cm)	109.5 (106.2, 112.8)	109.5 (106.2, 112.8)	0.01 (-2.16, 2.18)	**0.99**	111.5 (108.2, 114.7)	110.2 (106.8, 113.6)	-1.28 (-3.53, 0.98)	**0.27**	-1.29 (-4.42, 1.85)	**0.42**

In-person Cohort	CMT Pre (n=25)	CMT Post (n=24)	ΔCMT	ΔCMT *p* value	TRE Pre (n=25)	TRE Post (n=22)	ΔTRE	ΔTRE *p* value	Difference between groups	*p* value
Waist to Hip Ratio	0.980 (0.957, 1.004)	0.970 (0.946, 0.994)	-0.0107 (-0.0287, 0.0074)	**0.25**	0.953 (0.929, 0.977)	0.948 (0.924, 0.973)	-0.0047 (-0.0234, 0.0140)	**0.62**	0.0060 (-0.0200, 0.0320)	**0.65**
Bicep Circumference (cm)	35.4 (34.4, 36.4)	35.3 (34.3, 36.4)	-0.04 (-0.46, 0.38)	**0.86**	35.6 (34.6, 36.6)	35.3 (34.2, 36.3)	-0.30 (-0.74, 0.14)	**0.19**	-0.26 (-0.87, 0.35)	**0.41**
Thigh Circumference (cm)	57.5 (55.9, 59.2)	57.8 (56.2, 59.5)	0.27 (-0.37, 0.90)	**0.41**	57.7 (56.1, 59.4)	57.6 (55.9, 59.2)	-0.16 (-0.82, 0.51)	**0.64**	-0.42 (-1.35, 0.50)	**0.37**
Handgrip Strength (kg)	30.8 (26.8, 34.8)	31.1 (27.1, 35.1)	0.31 (-1.21, 1.83)	**0.69**	28.3 (24.3, 32.3)	28.8 (24.8, 32.8)	0.49 (-1.09, 2.08)	**0.54**	0.18 (-2.02, 2.38)	**0.87**
Leg Extension Peak Torque (ft-lbs)	109.3 (97.1, 121.4)	100.9 (88.5, 113.3)	-8.39 (-17.60, 0.81)	**0.07**	105.8 (93.6, 117.9)	105.9 (93.4, 118.4)	0.15 (-9.24, 9.53)	**0.98**	8.54 (-4.60, 21.69)	**0.20**
Respiratory Quotient	0.741 (0.717, 0.765)	0.776 (0.752, 0.801)	0.0348 (0.0119, 0.0577)	**0.003****	0.765 (0.741, 0.789)	0.767 (0.742, 0.792)	0.0028 (-0.0209, 0.0265)	**0.82**	-0.0320 (-0.0649, 0.0009)	**0.06**
Resting Metabolic Rate (kcal/day) [&]	1909.7 (1781.2, 2038.2)	1866.6 (1737.6, 1995.6)	-43.1 (-104.2, 18.0)	**0.17**	1920.4 (1791.9, 2048.9)	1892.3 (1762.0, 2022.5)	-28.1 (-91.8, 35.5)	**0.39**	15.0 (-108.1, 138.0)	**0.74**
Total Energy Expenditure (kcal/day) [&,g]	2772.1 (2563.4, 2980.7)	2644.7 (2436.1, 2853.4)	-127.3 (-230.7, -23.9)	**0.016***	2718.3 (2500.4, 2936.3)	2540.5 (2322.6, 2758.4)	-177.9 (-285.9, -69.9)	**0.001****	-50.6 (-259.2, 158.1)	**0.51**

Table 3.4. Insulin and glucose homeostasis in in-person subjects. All data is presented as means (95% confidence interval). For secondary outcome measures, Bonferroni-corrected confidence intervals are presented and Bonferroni-adjusted critical alpha of 0.006 is used. For data with statistical outliers, winsorised data was used to generate *p* value.
HbA1C, hemoglobin A1C; HOMA-IR, homeostatic model assessment of insulin resistance.
[&]secondary outcome using a Bonferroni-corrected alpha of 0.006 and presenting 99.7% CI for between group differences

	CMT Pre (n=25)	CMT Post (n=24)	ΔCMT	ΔCMT *p* value	TRE Pre (n=25)	TRE Post (n=22)	ΔTRE	ΔTRE *p* value	Difference between groups	*p* value
Glucose (mg/dL) [&]	93.9 (90.3, 97.5)	94.2 (90.5, 97.8)	0.29 (-2.41, 3.00)	**0.83**	91.7 (88.1, 95.3)	90.6 (86.9, 94.3)	-1.06 (-3.87, 1.75)	**0.46**	-1.35 (-5.25, 2.54)	**0.50**
Insulin (mU/L) [&]	14.7 (11.3, 18.0)	14.8 (11.5, 18.2)	0.19 (-1.58, 1.96)	**0.83**	12.4 (9.0, 15.7)	11.9 (8.5, 15.3)	-0.50 (-2.34, 1.35)	**0.60**	-0.69 (-3.25, 1.86)	**0.60**
HbA1C (%)[&]	5.30 (5.16, 5.44)	5.29 (5.16, 5.43)	-0.006 (-0.057, 0.044)	**0.81**	5.28 (5.14, 5.41)	5.25 (5.12, 5.39)	-0.024 (-0.077, 0.029)	**0.37**	-0.018 (-0.120, 0.084)	**0.63**
HOMA-IR	3.41 (2.62, 4.21)	3.50 (2.70, 4.30)	0.085 (-0.328, 0.498)	**0.69**	2.81 (2.02, 3.61)	2.65 (1.85, 3.46)	-0.160 (-0.590, 0.270)	**0.47**	-0.245 (-0.841, 0.351)	**0.42**

Table 3.5. Cardiovascular risk measurements in in-person subjects. All data is presented as means (95% confidence interval). For data with statistical outliers, winsorised data was used to generate *p* value.
HDL, high-density lipoprotein; LDL, low-density lipoprotein.
*within-group *p* value less than 0.05

	CMT Pre (n=25)	CMT Post (n=24)	ΔCMT	ΔCMT *p* value	TRE Pre (n=25)	TRE Post (n=22)	ΔTRE	ΔTRE *p* value	Difference between groups	*p* value
Systolic Blood Pressure (mmHg)	122.6 (119.0, 126.2)	118.7 (115.1, 122.4)	-3.9 (-7.6, -0.1)	**0.042***	119.8 (116.2, 123.5)	118.1 (114.3, 122.0)	-1.7 (-5.5, 2.2)	**0.39**	2.2 (-3.2, 7.5)	**0.43**
Diastolic Blood Pressure (mmHg)	74.6 (70.9, 78.4)	71.6 (67.8, 75.4)	-3.0 (-6.9, 0.9)	**0.13**	76.9 (73.2, 80.7)	72.8 (68.9, 76.8)	-4.1 (-8.1, -0.1)	**0.047***	-1.1 (-6.7, 4.5)	**0.71**
Total Cholesterol (mg/dL)	202.5 (187.9, 217.1)	203.5 (188.7, 218.2)	0.97 (-8.60, 10.55)	**0.84**	203.7 (189.1, 218.3)	200.1 (185.1, 215.1)	-3.56 (-13.52, 6.40)	**0.48**	-4.53 (-18.34, 9.29)	**0.52**
HDL (mg/dL)	50.1 (44.8, 55.5)	50.7 (45.4, 56.1)	0.61 (-1.72, 2.94)	**0.61**	54.7 (49.4, 60.1)	54.0 (48.6, 59.4)	-0.72 (-3.16, 1.71)	**0.56**	-1.33 (-4.70, 2.04)	**0.44**
LDL (mg/dL)	126.4 (114.9, 138.0)	124.2 (112.6, 135.7)	-2.24 (-10.14, 5.66)	**0.58**	122.1 (110.7, 133.6)	122.7 (111.0, 134.5)	0.60 (-7.46, 8.66)	**0.88**	2.84 (-8.45, 14.13)	**0.62**
Triglycerides (mg/dL)	133.4 (108.3, 158.4)	136.0 (110.8, 161.3)	2.67 (-13.88, 19.21)	**0.75**	127.2 (102.1, 152.2)	116.9 (91.2, 142.6)	-10.27 (-27.48, 6.94)	**0.24**	-12.94 (-36.81, 10.93)	**0.29**

Table 3.6. MOCACARE at home blood pressure measurements in total cohort. All data is presented as means (95% confidence interval). For data with statistical outliers, winsorised data was used to generate means, confidence intervals, and *p* values.
*within-group *p* value less than 0.05
**within group *p* value less than 0.01

	CMT Pre (n=23)	CMT Post (n=23)	ΔCMT	ΔCMT *p* value	TRE Pre (n=16)	TRE Post (n=16)	ΔTRE	ΔTRE *p* value	Difference between groups	*p* value
Systolic Blood Pressure (mmHg)	129.7 (124.9, 134.4)	124.3 (119.9, 128.7)	-5.4 (-9.1, -1.6)	**0.005****	126.5 (120.9, 132.1)	124.0 (118.8, 129.2)	-2.51 (-7.0, 2.0)	**0.28**	2.87 (-3.0, 8.8)	**0.34**
Diastolic Blood Pressure (mmHg)	81.0 (77.9, 84.1)	78.9 (75.9, 81.8)	-2.1 (-4.4, 0.2)	**0.07**	78.9 (75.2, 82.5)	75.3 (71.8, 78.8)	-3.6 (-6.3, -0.8)	**0.011***	-1.5 (-5.0, 2.1)	**0.42**

Table 3.7. Other blood markers in in-person cohort. All data is presented as means (95% confidence interval). For data with statistical outliers, winsorised data was used to generate *p* value.
GGT, gamma-glutamyl transferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALK, alkaline phosphatase; AMY, amylase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; Cre, creatinine; CPK, creatine phosphokinase; TP, total protein; BHBA, beta hydroxy butyrate; CO_2, carbon dioxide; Ca, calcium; Cl, chloride; Fe, iron; Hb, hemoglobin; K, potassium; Mg, magnesium; Na, sodium; Phos, phosphate.
*within-group *p* value less than 0.05
**within group *p* value less than 0.01
#between group *p* value less than 0.05
##between group *p* value less than 0.01

	CMT Pre (n=25)	CMT Post (n=24)	ΔCMT	ΔCMT *p* value	TRE Pre (n=25)	TRE Post (n=22)	ΔTRE	ΔTRE *p* value	Difference between groups	*p* value
GGT (U/L)	25.9 (19.9, 31.9)	23.7 (17.7, 29.6)	-2.22 (-3.93, -0.50)	**0.011***	24.0 (18.0, 30.0)	22.1 (16.2, 28.1)	-1.86 (-3.65, -0.07)	**0.042***	0.36 (-2.12, 2.84)	**0.78**
ALT (U/L)	34.0 (27.3, 40.6)	30.6 (23.9, 37.3)	-3.38 (-5.63, -1.13)	**0.003****	30.5 (23.8, 37.2)	28.1 (21.4, 34.8)	-2.40 (-4.75, -0.06)	**0.045***	0.98 (-2.27, 4.23)	**0.55**
AST (U/L)	28.1 (24.8, 31.4)	26.5 (23.2, 29.8)	-1.59 (-3.56, 0.38)	**0.11**	26.5 (23.2, 29.8)	25.7 (22.4, 29.1)	-0.77 (-2.82, 1.29)	**0.46**	0.82 (-2.02, 3.67)	**0.57**
ALK (U/L)	58.3 (51.5, 65.1)	56.9 (50.1, 63.7)	-1.37 (-3.60, 0.86)	**0.23**	57.8 (51.0, 64.6)	56.7 (49.9, 63.6)	-1.09 (-3.41, 1.24)	**0.36**	0.28 (-2.94, 3.51)	**0.86**
AMY (U/L)	61.3 (53.3, 69.3)	62.2 (54.2, 70.3)	0.93 (-3.42, 5.28)	**0.68**	57.2 (49.2, 65.2)	57.9 (49.8, 66.1)	0.71 (-3.82, 5.24)	**0.76**	-0.22 (-6.50, 6.06)	**0.95**
LDH (U/L)	155.2 (144.3, 166.0)	145.2 (134.3, 156.2)	-9.92 (-19.80, -0.04)	**0.049***	149.8 (139.0, 160.7)	154.3 (143.0, 165.6)	4.45 (-5.79, 14.68)	**0.39**	14.37 (0.14, 28.60)	**0.048#**
Total Bilirubin (mg/dL)	0.790 (0.694, 0.886)	0.762 (0.664, 0.859)	-0.0284 (-0.1113, 0.0544)	**0.50**	0.856 (0.760, 0.952)	0.829 (0.729, 0.929)	-0.0267 (-0.1126, 0.0592)	**0.54**	0.0018 (-0.1176, 0.1211)	**0.98**
BUN (mg/dL)	16.4 (15.0, 17.8)	16.5 (15.1, 17.9)	0.11 (-1.03, 1.24)	**0.85**	14.9 (13.5, 16.3)	14.8 (13.3, 16.2)	-0.10 (-1.28, 1.08)	**0.87**	-0.21 (-1.84, 1.43)	**0.80**

	CMT Pre (n=25)	CMT Post (n=24)	ΔCMT	ΔCMT *p* value	TRE Pre (n=25)	TRE Post (n=22)	ΔTRE	ΔTRE *p* value	Difference between groups	*p* value
CRE (mg/dL)	0.864 (0.794, 0.934)	0.845 (0.774, 0.915)	-0.0195 (-0.0548, 0.0158)	**0.28**	0.854 (0.784, 0.924)	0.841 (0.770, 0.912)	-0.0127 (-0.0495, 0.0241)	**0.50**	0.0068 (-0.0442, 0.0578)	**0.79**
CPK (U/L)	140.2 (105.3, 175.1)	145.5 (110.2, 180.7)	5.28 (-21.95, 32.50)	**0.70**	142.1 (107.2, 177.0)	128.0 (91.9, 164.0)	-14.15 (-42.42, 14.11)	**0.33**	-19.43 (-58.68, 19.82)	**0.33**
Albumin (g/dL)	4.25 (4.14, 4.35)	4.22 (4.11, 4.32)	-0.033 (-0.119, 0.053)	**0.45**	4.21 (4.10, 4.31)	4.22 (4.11, 4.33)	0.013 (-0.077, 0.102)	**0.78**	0.046 (-0.078, 0.170)	**0.47**
TP (g/dL)	7.00 (6.85, 7.16)	6.93 (6.77, 7.08)	-0.079 (-0.211, 0.053)	**0.24**	6.98 (6.83, 7.14)	6.98 (6.83, 7.14)	-0.001 (-0.138, 0.135)	**0.99**	0.077 (-0.112, 0.267)	**0.42**
BHBA (mmol/L)	0.052 (0.039, 0.065)	0.061 (0.048, 0.075)	0.0089 (-0.0072, 0.0250)	**0.28**	0.041 (0.028, 0.054)	0.051 (0.037, 0.065)	0.0098 (-0.0067, 0.0264)	**0.24**	0.0009 (-0.0221, 0.0240)	**0.94**
CO_2 (mEq/L)	24.5 (23.8, 25.2)	24.3 (23.6, 25.0)	-0.15 (-0.78, 0.48)	**0.64**	24.6 (23.9, 25.3)	24.2 (23.5, 24.9)	-0.44 (-1.09, 0.22)	**0.19**	-0.29 (-1.20, 0.62)	**0.53**
Uric Acid (mg/dL)	5.59 (5.16, 6.02)	5.99 (5.56, 6.43)	0.401 (0.096, 0.706)	**0.01***	5.94 (5.52, 6.37)	5.75 (5.31, 6.19)	-0.194 (-0.511, 0.123)	**0.23**	-0.595 (-1.034, -0.155)	**0.008**##
Ca (mg/dL)	9.24 (9.12, 9.37)	9.22 (9.10, 9.35)	-0.022 (-0.109, 0.065)	**0.62**	9.28 (9.15, 9.40)	9.30 (9.17, 9.43)	0.027 (-0.063, 0.118)	**0.56**	0.049 (-0.076, 0.174)	**0.44**
Cl (mEq/L)	104.0 (103.4, 104.7)	104.1 (103.4, 104.8)	0.08 (-0.63, 0.78)	**0.83**	103.9 (103.3, 104.6)	104.1 (103.4, 104.9)	0.21 (-0.52, 0.94)	**0.58**	0.13 (-0.88, 1.14)	**0.80**
Fe (mcg/dL)	110.5 (98.2, 122.7)	102.1 (89.7, 114.6)	-8.32 (-21.49, 4.84)	**0.22**	93.4 (81.1, 105.7)	93.4 (80.4, 106.3)	-0.03 (-13.62, 13.56)	**1.00**	8.29 (-10.62, 27.21)	**0.39**
Hb (g/dL)	15.3 (14.6, 15.9)	15.6 (15.0, 16.3)	0.36 (-0.38, 1.10)	**0.34**	16.0 (15.4, 16.7)	15.8 (15.1, 16.5)	-0.18 (-0.94, 0.59)	**0.65**	-0.54 (-1.60, 0.53)	**0.32**
K (mEq/L)	4.12 (4.00, 4.23)	4.10 (3.99, 4.22)	-0.011 (-0.117, 0.096)	**0.84**	4.12 (4.00, 4.23)	4.17 (4.05, 4.29)	0.050 (-0.060, 0.161)	**0.37**	0.061 (-0.093, 0.214)	**0.44**

	CMT Pre (n=25)	CMT Post (n=24)	ΔCMT	ΔCMT *p* value	TRE Pre (n=25)	TRE Post (n=22)	ΔTRE	ΔTRE *p* value	Difference between groups	*p* value
Mg (mg/dL)	2.09 (2.03, 2.15)	2.07 (2.01, 2.13)	-0.015 (-0.057, 0.028)	**0.50**	2.07 (2.01, 2.13)	2.06 (2.00, 2.12)	-0.007 (-0.051, 0.038)	**0.77**	0.008 (-0.053, 0.069)	**0.80**
Na (mEq/L)	137.5 (136.9, 138.1)	137.8 (137.1, 138.4)	0.24 (-0.34, 0.82)	**0.42**	137.7 (137.1, 138.3)	137.7 (137.1, 138.3)	0.01 (-0.60, 0.61)	**0.99**	-0.23 (-1.07, 0.60)	**0.59**
Phos (mg/dL)	3.22 (3.04, 3.39)	3.37 (3.19, 3.54)	0.153 (-0.034, 0.340)	**0.11**	3.61 (3.44, 3.78)	3.61 (3.42, 3.79)	-0.003 (-0.196, 0.190)	**0.98**	-0.156 (-0.424, 0.112)	**0.25**

Table 3.8. Subjective scores of sleep quality and rewards-based eating habits. All data is presented as means (95% confidence interval). For data with statistical outliers, winsorised data was used to generate means, confidence intervals, and *p* values.
In scoring the PSQI, seven component scores are derived, each scored 0 (no difficulty) to 3 (severe difficulty). The component scores are summed to produce a global score (range 0 to 21). Higher scores indicate worse sleep quality.
For the RED Scale, participants read statements about food and eating. On a scale of 1- "Not at all like me" to 5- "Exactly like me", participants rated how they agreed with the statement. Score ranges from 9-45; higher scores indicate higher rewards-based eating drive.
PSQI, Pittsburg Sleep Quality Index; RED Scale, Rewards-based Eating Drive Scale.

	CMT Pre	CMT Post	ΔCMT	ΔCMT *p* value	TRE Pre	TRE Post	ΔTRE	ΔTRE *p* value	Difference between groups	*P* value
PSQI Score	4.63 (4.02, 5.24) (n=43)	4.26 (3.65, 4.87) (n=43)	-0.366 (-0.820, 0.087)	**0.11**	4.62 (4.02, 5.22) (n=46)	4.60 (3.99, 5.20) (n=44)	-0.018 (-0.455, 0.420)	**0.94**	0.349 (-0.281, 0.979)	**0.28**
RED Scale Score	21.8 (19.6, 23.9) (n=41)	21.1 (18.8, 23.4) (n=29)	-0.68 (-2.27, 0.91)	**0.40**	23.1 (21.0, 25.2) (n=44)	22.0 (19.8, 24.3) (n=28)	-1.11 (-2.68, 0.45)	**0.16**	-0.43 (-2.66, 1.79)	**0.70**

Table 3.9. Sleep and activity measures from Oura ring in subset of in-person cohort. All data is presented as means (95% confidence interval). For data with statistical outliers, winsorised data was used to generate means, confidence intervals, and *p* values.
*within-group *p* value less than 0.05
**within group *p* value less than 0.01
***within group *p* value less than 0.001
#between group *p* value less than 0.05

	CMT Pre	CMT Post	ΔCMT	ΔCMT *p* value	TRE Pre	TRE Post	ΔTRE	ΔTRE *p* value	Difference between groups	*P* value
	n=17 subjects analyzed				n=17 subjects analyzed					
Activity Balance Score	84.0 (79.9, 88.0)	84.1 (78.3, 90.0)	0.18 (-6.66, 7.03)	**0.96**	85.2 (81.1, 89.2)	89.0 (83.4, 94.7)	3.89 (-2.76, 10.53)	**0.25**	3.71 (-5.84, 13.25)	**0.45**
Activity Burn	401.1 (325.0, 477.2)	380.7 (292.6, 468.9)	-20.35 (-77.45, 36.75)	**0.48**	394.2 (318.1, 470.2)	289.9 (203.9, 375.8)	-104.29 (-158.60, -49.98)	**<0.001*** **	-83.94 (-162.74, -5.14)	**0.037#**
Activity Score	76.5 (70.4, 82.6)	72.6 (65.8, 79.4)	-3.89 (-9.74, 1.97)	**0.19**	80.2 (74.1, 86.3)	70.7 (64.1, 77.4)	-9.44 (-15.07, -3.82)	**<0.001*** **	-5.56 (-13.68, 2.56)	**0.18**
Average HRV	35.6 (25.8, 45.4)	30.6 (22.0, 39.2)	-5.04 (-13.29, 3.21)	**0.23**	39.9 (30.2, 49.7)	42.9 (34.5, 51.4)	3.00 (-5.03, 11.03)	**0.46**	8.04 (-3.47, 19.55)	**0.17**
Average MET	1.43 (1.37, 1.48)	1.42 (1.36, 1.48)	-0.007 (-0.046, 0.031)	**0.72**	1.42 (1.37, 1.48)	1.36 (1.30, 1.42)	-0.061 (-0.097, -0.024)	**0.001*** **	-0.054 (-0.107, 0.000)	**0.049#**
Average Resting Heart Rate	65.6 (61.9, 69.3)	65.9 (61.9, 69.9)	0.31 (-1.75, 2.36)	**0.77**	65.2 (61.5, 68.9)	64.9 (61.0, 68.9)	-0.25 (-2.25, 1.75)	**0.81**	-0.56 (-3.43, 2.31)	**0.70**
Awake Time	3829.7 (3285.1, 4374.3)	3414.5 (2710.7, 4118.3)	-415.26 (-1130.07, 299.55)	**0.25**	3270.9 (2727.9, 3813.9)	4169.4 (3477.9, 4861.0)	898.53 (195.68, 1601.37)	**0.012***	1313.79 (311.32, 2316.26)	**0.01#**
Daily Movement	7054.5 (5659.7, 8449.2)	6625.8 (4965.8, 8285.8)	-428.70 (-1542.25, 684.85)	**0.45**	7196.8 (5803.7, 8590.0)	5094.7 (3476.0, 6713.4)	-2102.14 (-3162.54, -1041.73)	**<0.001*** **	-1673.44 (-3211.11, -135.76)	**0.033#**
Deep Sleep Score	65.6 (54.5, 76.8)	66.7 (56.6, 76.8)	1.08 (-8.04, 10.20)	**0.82**	78.8 (67.7, 89.9)	83.0 (73.0, 93.0)	4.22 (-4.74, 13.19)	**0.36**	3.14 (-9.64, 15.93)	**0.63**

	CMT Pre	CMT Post	ΔCMT	ΔCMT *p* value	TRE Pre	TRE Post	ΔTRE	ΔTRE *p* value	Difference between groups	*P* value
Deep Sleep Time	3827.3 (2722.7, 4931.9)	3669.7 (2603.2, 4736.2)	-157.57 (-1000.79, 685.65)	**0.71**	5106.5 (4002.6, 6210.4)	5467.7 (4416.1, 6519.4)	361.21 (-463.97, 1186.39)	**0.39**	518.79 (-661.02, 1698.59)	**0.39**
High Activity Time	1.21 (0.63, 1.78)	0.53 (-0.07, 1.13)	-0.677 (-1.061, -0.292)	**<0.001*** **	1.25 (0.67, 1.83)	0.98 (0.41, 1.56)	-0.265 (-0.627, 0.097)	**0.15**	0.412 (-0.116, 0.940)	**0.13**
Inactive Time	514.6 (451.4, 577.9)	472.1 (395.6, 548.6)	-42.52 (-84.55, -0.49)	**0.047***	476.0 (412.8, 539.1)	387.1 (312.2, 462.0)	-88.90 (-128.60, -49.19)	**<0.001*** **	-46.38 (-104.20, 11.44)	**0.12**
Light Sleep Time	13525.6 (12043.5, 15007.7)	14788.1 (12824.7, 16751.5)	1262.53 (-670.62, 3195.68)	**0.20**	14099.2 (12619.2, 15579.2)	14750.8 (12835.0, 16666.5)	651.54 (-1229.26, 2532.33)	**0.50**	-610.99 (-3308.12, 2086.13)	**0.66**
Long Periods of Inactivity	0.431 (0.257, 0.606)	0.454 (0.231, 0.678)	0.0230 (-0.1457, 0.1916)	**0.79**	0.538 (0.364, 0.712)	0.468 (0.257, 0.680)	-0.0697 (-0.2272, 0.0879)	**0.39**	-0.0926 (-0.3235, 0.1382)	**0.43**
Low Activity Time	266.3 (222.2, 310.4)	254.5 (208.9, 300.1)	-11.85 (-44.74, 21.04)	**0.48**	233.5 (189.5, 277.6)	184.9 (140.0, 229.8)	-48.62 (-80.28, -16.96)	**0.003** **	-36.77 (-82.42, 8.88)	**0.11**
Lowest Resting Heart Rate	56.8 (53.2, 60.4)	57.6 (53.8, 61.4)	0.80 (-1.02, 2.61)	**0.39**	56.4 (52.8, 60.0)	56.0 (52.2, 59.8)	-0.42 (-2.19, 1.35)	**0.64**	-1.22 (-3.76, 1.32)	**0.35**
Medium Activity Time	33.6 (24.6, 42.6)	31.7 (19.7, 43.8)	-1.88 (-10.52, 6.76)	**0.67**	39.9 (30.9, 48.9)	28.6 (16.9, 40.3)	-11.22 (-19.45, -2.99)	**0.008** **	-9.35 (-21.28, 2.59)	**0.12**
Meet Daily Targets Score	61.1 (45.9, 76.3)	54.8 (39.4, 70.2)	-6.33 (-20.09, 7.44)	**0.37**	59.4 (44.2, 74.6)	35.3 (20.3, 50.2)	-24.13 (-37.39, -10.86)	**<0.001*** **	-17.80 (-36.92, 1.32)	**0.07**
Move Every Hour Score	97.6 (96.7, 98.6)	97.5 (96.3, 98.8)	-0.08 (-1.03, 0.87)	**0.87**	97.1 (96.2, 98.1)	97.5 (96.3, 98.7)	0.35 (-0.53, 1.24)	**0.43**	0.43 (-0.86, 1.73)	**0.51**
Non-wear Time	95.1 (37.1, 153.1)	124.8 (50.5, 199.1)	29.73 (-31.78, 91.24)	**0.34**	120.7 (62.8, 178.7)	230.2 (157.6, 302.9)	109.50 (50.46, 168.53)	**<0.001*** **	79.76 (-5.49, 165.02)	**0.07**
Previous Day Activity Score	79.7 (73.9, 85.4)	77.0 (70.1, 83.9)	-2.66 (-7.38, 2.05)	**0.27**	75.3 (69.6, 81.1)	70.5 (63.7, 77.4)	-4.78 (-9.38, -0.19)	**0.041***	-2.12 (-8.70, 4.47)	**0.53**

	CMT Pre	CMT Post	ΔCMT	ΔCMT *p* value	TRE Pre	TRE Post	ΔTRE	ΔTRE *p* value	Difference between groups	*P* value
Previous Night Score	73.7 (69.2, 78.2)	69.1 (63.7, 74.6)	-4.55 (-10.36, 1.27)	**0.13**	74.3 (69.8, 78.7)	69.3 (64.1, 74.5)	-4.95 (-10.63, 0.73)	**0.09**	-0.41 (-8.53, 7.72)	**0.92**
Readiness Score	77.2 (74.6, 79.8)	76.1 (73.4, 78.8)	-1.08 (-3.88, 1.72)	**0.45**	79.4 (76.8, 82.0)	77.5 (74.9, 80.0)	-1.97 (-4.70, 0.77)	**0.16**	-0.88 (-4.80, 3.03)	**0.66**
Recovery Index Score	67.5 (61.8, 73.1)	63.5 (56.1, 70.9)	-4.01 (-11.75, 3.74)	**0.31**	74.2 (68.6, 79.8)	67.7 (60.7, 74.8)	-6.48 (-14.00, 1.05)	**0.09**	-2.47 (-13.27, 8.33)	**0.65**
Recovery Time Score	94.6 (89.4, 99.9)	93.8 (88.2, 99.3)	-0.86 (-4.90, 3.18)	**0.68**	97.7 (92.4, 102.9)	100.7 (95.3, 106.0)	3.00 (-0.86, 6.86)	**0.13**	3.86 (-1.73, 9.45)	**0.18**
REM Sleep Score	83.4 (74.9, 91.8)	79.7 (70.3, 89.1)	-3.71 (-9.63, 2.21)	**0.22**	80.8 (72.4, 89.2)	74.9 (65.6, 84.2)	-5.86 (-11.65, -0.07)	**0.047***	-2.15 (-10.43, 6.13)	**0.61**
REM Sleep Time	7722.6 (6381.6, 9063.6)	6180.8 (4842.3, 7519.3)	-1541.78 (-2935.64, -147.92)	**0.03***	6649.8 (5310.0, 7989.6)	5456.5 (4147.4, 6765.6)	-1193.30 (-2557.89, 171.29)	**0.09**	348.48 (-1602.15, 2299.11)	**0.73**
Respiratory Rate	15.3 (14.6, 16.0)	15.3 (14.6, 16.0)	0.02 (-0.29, 0.32)	**0.91**	15.4 (14.7, 16.1)	15.6 (14.9, 16.3)	0.18 (-0.12, 0.48)	**0.23**	0.16 (-0.26, 0.59)	**0.45**
Resting Heart Rate Score	82.4 (78.3, 86.4)	79.1 (74.1, 84.2)	-3.24 (-9.32, 2.85)	**0.30**	84.9 (80.9, 88.9)	81.7 (76.9, 86.5)	-3.18 (-9.13, 2.76)	**0.29**	0.05 (-8.45, 8.56)	**0.99**
Restless Sleep	36.6 (30.5, 42.8)	35.9 (29.7, 42.2)	-0.68 (-3.82, 2.45)	**0.67**	38.2 (32.0, 44.3)	38.4 (32.2, 44.6)	0.21 (-2.85, 3.27)	**0.89**	0.90 (-3.49, 5.28)	**0.69**
Rest Time	472.9 (438.5, 507.3)	412.5 (355.3, 469.7)	-60.39 (-115.47, -5.31)	**0.032***	486.5 (452.2, 520.9)	383.3 (328.3, 438.2)	-103.25 (-155.98, -50.52)	**<0.001*****	-42.86 (-119.11, 33.40)	**0.27**
Sleep Balance Score	74.2 (68.9, 79.4)	78.4 (71.0, 85.8)	4.25 (-3.53, 12.04)	**0.28**	78.6 (73.3, 83.8)	77.4 (70.3, 84.6)	-1.17 (-8.69, 6.36)	**0.76**	-5.42 (-16.25, 5.40)	**0.33**
Sleep Efficiency	87.1 (85.4, 88.9)	88.0 (85.9, 90.1)	0.89 (-1.28, 3.06)	**0.42**	88.8 (87.1, 90.5)	86.1 (84.0, 88.2)	-2.68 (-4.82, -0.55)	**0.014***	-3.58 (-6.62, -0.53)	**0.021#**

	CMT Pre	CMT Post	ΔCMT	ΔCMT *p* value	TRE Pre	TRE Post	ΔTRE	ΔTRE *p* value	Difference between groups	*P* value
Sleep Efficiency Score	86.8 (83.6, 90.1)	88.6 (84.4, 92.7)	1.74 (-2.62, 6.09)	**0.43**	90.0 (86.7, 93.2)	84.8 (80.7, 88.8)	-5.22 (-9.50, -0.94)	**0.017***	-6.96 (-13.06, -0.85)	**0.026#**
Sleep Latency	673.8 (513.3, 834.4)	634.2 (449.3, 819.1)	-39.65 (-211.18, 131.88)	**0.65**	736.5 (576.4, 896.6)	767.1 (587.9, 946.2)	30.55 (-137.42, 198.52)	**0.72**	70.20 (-169.87, 310.27)	**0.57**
Sleep Latency Score	84.1 (81.7, 86.5)	85.2 (82.1, 88.3)	1.03 (-1.69, 3.74)	**0.46**	84.6 (82.2, 87.0)	81.7 (78.7, 84.7)	-2.94 (-5.57, -0.32)	**0.028***	-3.97 (-7.75, -0.19)	**0.039#**
Sleep Score	79.7 (76.3, 83.1)	78.5 (75.2, 81.9)	-1.17 (-4.29, 1.95)	**0.46**	79.7 (76.3, 83.1)	77.6 (74.4, 80.8)	-2.14 (-5.21, 0.93)	**0.17**	-0.97 (-5.34, 3.41)	**0.67**
Sleep Timing	14743.5 (13995.1, 15491.9)	14095.1 (13294.8, 14895.4)	-648.44 (-1375.71, 78.84)	**0.08**	14546.0 (13800.5, 15291.6)	14966.9 (14174.8, 15759.1)	420.91 (-296.90, 1138.71)	**0.25**	1069.34 (47.50, 2091.19)	**0.04#**
Sleep Timing Score	88.4 (79.5, 97.3)	80.4 (69.5, 91.3)	-8.04 (-17.63, 1.56)	**0.10**	66.5 (57.6, 75.4)	65.6 (55.0, 76.3)	-0.85 (-10.24, 8.54)	**0.86**	7.19 (-6.24, 20.62)	**0.29**
Sleep Tranquility Score	76.9 (71.0, 82.9)	77.5 (71.1, 83.9)	0.53 (-3.09, 4.15)	**0.77**	78.0 (72.1, 84.0)	74.5 (68.2, 80.8)	-3.55 (-7.09, -0.01)	**0.049***	-4.08 (-9.15, 0.98)	**0.11**
Stay Active Score	73.9 (69.5, 78.4)	75.0 (70.2, 79.8)	1.05 (-2.44, 4.55)	**0.55**	77.2 (72.8, 81.6)	80.5 (75.7, 85.2)	3.24 (-0.10, 6.57)	**0.06**	2.18 (-2.65, 7.01)	**0.38**
Steps	8871.4 (7194.2, 10548.6)	8613.9 (6708.9, 10518.9)	-257.48 (-1756.20, 1241.23)	**0.74**	8555.4 (6879.5, 10231.4)	6056.6 (4189.0, 7924.1)	-2498.89 (-3939.91, -1057.88)	**<0.001*** **	-2241.41 (-4320.51, -162.31)	**0.035#**
Target Calories	402.9 (359.8, 446.1)	415.4 (371.6, 459.1)	12.45 (-20.48, 45.38)	**0.46**	425.6 (382.5, 468.7)	426.6 (383.6, 469.7)	1.07 (-30.65, 32.79)	**0.95**	-11.38 (-57.11, 34.34)	**0.63**
Temperature Deviation	-0.061 (-0.111, -0.010)	-0.088 (-0.162, -0.013)	-0.0271 (-0.1020, 0.0477)	**0.48**	-0.020 (-0.069, 0.030)	-0.007 (-0.079, 0.065)	0.0125 (-0.0607, 0.0856)	**0.74**	0.0396 (-0.0650, 0.1442)	**0.46**
Temperature Score	89.5 (86.9, 92.0)	88.4 (83.9, 92.8)	-1.12 (-6.19, 3.95)	**0.66**	89.2 (86.7, 91.7)	85.9 (81.6, 90.2)	-3.30 (-8.27, 1.67)	**0.19**	-2.18 (-9.28, 4.92)	**0.55**

	CMT Pre	CMT Post	ΔCMT	ΔCMT *p* value	TRE Pre	TRE Post	ΔTRE	ΔTRE *p* value	Difference between groups	*P* value
Total Bedtime	29310.3 (27908.0, 30712.6)	28048.3 (26486.0, 29610.6)	-1261.99 (-2803.55, 279.56)	**0.11**	29103.3 (27706.1, 30500.6)	29774.0 (28230.8, 31317.3)	670.68 (-851.56, 2192.92)	**0.39**	1932.67 (-233.80, 4099.15)	**0.08**
Total Burn	2611.2 (2430.3, 2792.0)	2626.2 (2457.5, 2794.9)	15.00 (-106.66, 136.67)	**0.81**	2590.0 (2409.3, 2770.8)	2532.7 (2367.2, 2698.2)	-57.33 (-174.56, 59.90)	**0.34**	-72.33 (-241.29, 96.62)	**0.40**
Total Sleep Score	78.7 (74.0, 83.4)	76.6 (71.5, 81.6)	-2.13 (-6.94, 2.68)	**0.39**	79.1 (74.5, 83.8)	77.5 (72.4, 82.5)	-1.66 (-6.41, 3.08)	**0.49**	0.47 (-6.29, 7.22)	**0.89**
Total Sleep Time	25305.6 (24042.3, 26569.0)	24568.6 (23321.1, 25816.1)	-737.01 (-1955.14, 481.12)	**0.24**	25840.0 (24580.9, 27099.2)	25469.4 (24234.4, 26704.3)	-370.67 (-1575.01, 833.66)	**0.55**	366.34 (-1346.64, 2079.31)	**0.68**
Training Frequency Score	57.6 (41.7, 73.5)	49.4 (29.8, 69.1)	-8.20 (-25.24, 8.84)	**0.35**	76.7 (60.9, 92.6)	54.4 (35.3, 73.5)	-22.31 (-38.66, -5.96)	**0.007****	-14.11 (-37.72, 9.51)	**0.24**
Training Volume Score	74.8 (63.9, 85.6)	63.6 (47.7, 79.5)	-11.21 (-23.00, 0.58)	**0.06**	81.3 (70.4, 92.1)	63.2 (47.6, 78.8)	-18.07 (-29.37, -6.78)	**0.002****	-6.87 (-23.19, 9.46)	**0.41**

Chapter 4- DISCUSSION

4.1 Summary

Here we present results from a 12-week prospective randomized controlled trial of individuals with overweight and obesity comparing the prescription of a TRE schedule to a CMT schedule. TRE is attractive as a weight-loss option in that it does not require tedious, unsustainable, and time-consuming methods such as calorie-counting and does not require any special instructions or adherence to complicated diets. Self-reported adherence to the TRE schedule was high (see Figure 3.2A); however, while there was a modest decrease in weight in the TRE group, there was no significant difference in weight loss between TRE and CMT groups. Despite weight loss, there were no significant changes in fat mass, fasting insulin, glucose, or HbA1C, or blood lipids. However, lean body mass decreased significantly in the TRE group. Moreover, appendicular lean mass and appendicular lean mass index decreased significantly in the TRE group, and this change was significant compared to the CMT group. There were no significant changes in RMR, but there was a significant decrease in total energy expenditure (TEE) in both groups (but no difference between groups). Overall, we found TRE led to a modest reduction in weight that was mostly due to loss of lean mass.

4.2 Effect of Time-Restricted Eating on Body Weight

The principle finding of this study is that TRE did not lead to significant weight loss as compared to the control group. Most humans eat throughout the majority of their waking hours. A study of 156 subjects found that the median daily eating duration was nearly 15 hours per day, and less than 10% of subjects had an eating window less than 12 hours per day (9). We prescribed an 8-hour eating window and intentionally did not prescribe calorie or macronutrient guidance with the goal

of offering a simple and easy to follow real-world recommendation that would substantially reduce daily eating duration for most adults. We selected the 12:00pm to 8:00pm eating window in an attempt to improve adherence under the assumption that skipping dinner and evening events is less compatible with modern culture. Self-reported adherence was high in our study, but there was no significant difference in estimated energy intake. It is likely that subjects experienced a compensatory increase in food intake during their eating window to offset the caloric deficit imposed by fasting, which has been observed in mice fed a TRF protocol (5). Additionally, Dhurandhar et al. have demonstrated that a recommendation to skip or eat breakfast does not affect weight outcomes in subjects trying to lose weight (33). However, the results presented here contradict previous reports describing the beneficial effects of TRE on weight loss and other outcomes such as fat mass, fasting glucose and insulin, and cardiovascular risk markers (10, 13, 14, 34).

Wilkinson et al. found that TRE leads to ~3% weight loss and improvements in cardiovascular risk markers in patients with Metabolic Syndrome over 12 weeks (13). The eligibility criteria excluded participants whose daily eating window was less than 14 hours. We excluded subjects who did not regularly consume breakfast, but we did not screen participants based on eating window times. It is possible that the change in daily eating window times in our study was not sufficient to provide benefits of TRE. However, the Wilkinson study was a small (n=19), single-arm study and, importantly, did not have a control group.

The prescribed 8-hour eating window may not be optimal for the metabolic advantages of TRE. Sutton et al. performed a 5-week randomized crossover trial comparing early TRE (eTRE: 6 hour eating window with dinner before 3:00pm) to a control diet (12-hour eating window) (14). The study staff provided all food to study participants, and the diets were isocaloric and eucaloric.

Body weight was maintained throughout the study, yet they found improved glycemic control and improvements in cardiovascular risk markers in the eTRE group. It is important to note that the eTRE subjects fasted for approximately 18 hours prior to clinic visits while the control arm only fasted for 12 hours. In our study, all participants began fasting at 8pm the night prior to clinic visits regardless of study group.

4.3 Effect of Time-Restricted Eating on Lean Mass and Other Body Composition Parameters

Perhaps the most striking and unexpected result of this study was the significant reduction in lean mass in the TRE group. In the in-person cohort, the average weight loss in the TRE group was 1.70kg. Of this, 1.10kg (~65% of weight lost) was due to lean mass; only 0.5kg of weight loss was due to fat mass. Loss of lean mass during weight loss is not unexpected; many weight loss studies have demonstrated that lean mass loss accounts for 20-30% of total weight loss (35-41). However, the proportion of lean mass loss in this study (~65%) far exceeds the normal range of 20-30% (41). Additionally, there was a highly significant between group difference in appendicular lean mass. Appendicular lean mass is correlated with nutritional and physical status, and reduced appendicular lean mass leads to weakness, disability, and impaired quality of life (42-44). These data suggest that TRE might lead to a reduction in skeletal muscle mass. The degree of lean mass loss in the TRE group is notable. This is potentially concerning for elderly or other patient populations at risk for sarcopenia as TRE could exacerbate muscle loss (45). Finally, the extent of lean mass loss during weight loss has been positively correlated to weight regain (46).

We are not the first to report that TRE impacts lean mass. Antoni et al. demonstrated that *ad libitum* feeding during TRE leads to reduced calorie intake and might also reduce protein intake (12); however, TRE's effects on lean mass is largely unexplored. In one study of resistance trained males, TRE subjects lost 0.2kg lean mass while the normal diet subjects gained 2.3kg of lean mass,

suggesting that TRE may have hindered lean mass gains. It is important to note that calorie and macronutrient intake was not prescribed, and subjects in the TRE group decreased self-reported calorie and protein intake by ~650kcal/day and 30g/day, respectively, on fasting days (47). A follow-up study showed that when calorie intake and protein intake were matched to pre-study consumption, no change in lean mass was observed (48). It has been shown that inadequate protein intake augments lean mass loss during weight loss (41). Mammalian target of rapamycin (mTor) is the primary regulator of muscle protein synthesis (MPS) (49). Protein intake elevates plasma amino acid levels, and amino acids (primarily leucine) activate mTor and lead to MPS (49, 50). High adenosine monophosphate kinase (AMPK) activity leads to mTor inhibition and therefore reduced muscle mass (51). Caloric deprivation and fasting can elevate AMPK activity (51, 52). It is possible that the reduced mTor activation due to reduced protein intake, paired with the increased AMPK activity due to fasting, exacerbates muscle loss during TRE. Together, this data highlights the importance of adequate protein consumption while adhering to a TRE diet and suggests that maintaining adequate protein intake may prevent excessive loss of lean mass while following a TRE diet.

The mechanism of lean mass loss in the TRE group is unknown; however, we hypothesize that it may relate to TRE-induced differences in the quantity and/or timing of protein consumption. Many studies have shown that adequate/excessive protein consumption during weight loss can mitigate losses in lean mass (35, 46, 53-56). NHANES data show that the majority of daily protein intake occurs during meals, and snacking accounts for a small portion of total daily protein intake (57). One study reported that some TRE subjects reported eating “less healthy” via increased consumption of convenience foods due to time restrictions with food preparation, and TRE subjects tended to eat less protein (effect size, -24.6g/day, p=0.06) (12). It is possible that TRE

subjects skipped breakfast and inadvertently increased snacking to compensate for the calories lost by skipping breakfast thereby decreasing their daily protein intake. If this hypothesis is correct, the loss of lean mass during TRE could be mitigated by increasing the number of meals within the eating window or consuming protein supplements (35, 46).

Recent studies have also suggested that timing of protein consumption may play a role in changes in lean mass (58-60). Mamerow et al. found an increase in 24-hour MPS in subjects who ate three evenly spaced meals containing 30g of protein each throughout the day as compared to subjects who skewed their protein intake to later in the day (10 gram breakfast, 15 gram lunch, 65 gram dinner) (59). Others have found conflicting results (56, 61). Lean mass flux depends on both MPS and muscle proteolysis, and it is unclear how daily protein distribution affects muscle proteolysis in subjects actively losing weight. This could also potentially point to a potential advantage of eTRE versus late TRE employed in this study. In fact, one RCT demonstrated a significant reduction in lean mass accompanied by weight loss in subjects following a TRE schedule (15). In this RCT, lean mass loss accounted for only 39% of weight loss compared to 68% in our study; however, calorie and macronutrient intake was not prescribed or recorded, and, importantly, the eating window timing was self-selected and tended to begin earlier than 12:00pm (as was assigned for the TREAT study) (15). Future studies should address how TRE affects protein synthesis and consumption and whether manipulating the quantity and/or timing of protein intake (or fasting) influences loss of lean mass.

TRE may result in decreased physical activity, which could be another potential mechanism to explain the loss in lean mass. Data from the Oura ring (n=32) demonstrates that daily step count decreased significantly in the TRE group but not in the CMT group, and the difference between groups was significant (Table 3.9). This reduction in daily step count is important as a reduction

is step count may lead to decreased fat-free mass, increased fat mass, and impaired insulin sensitivity (62-64). Whether physical activity decreased in the TRE group is unclear since the change in TEE is similar between groups, but future studies should investigate if and how TRE negatively impacts physical activity levels and how this influences lean muscle mass.

4.4 Effect of Time-Restricted Eating on Sleep

As an exploratory analysis, we utilized the Oura ring to determine if TRE affects sleep patterns. Previous work in animal models showed that TRF improves diurnal rhythms, which are inextricably linked to sleep (5, 8, 65, 66). Additionally, studies on Drosophila demonstrated that sleep patterns and total amount of sleep improved in TRF-fed flies compared to *ad libitum*-fed flies (67). Therefore, we hypothesized that TRE would lead to an improvement in sleep quality due the reported effects of TRE on improving diurnal rhythms. Unexpectedly, we found that TRE led to impaired sleep efficiency, sleep latency, and time awake in bed, suggesting that TRE may have adverse effects on sleep. Further studies are needed to validate this finding.

4.5 Strengths and Limitations

To date, this study is the first and only large, prospective randomized controlled trial designed to determine the effects of TRE on weight loss and metabolic health. The 12-week duration of this study is ample time to detect potential changes in weight loss in a cohort of 116 subjects who are overweight or obese. Strengths of this study include that it featured a simple real-world prescription-based intervention, which is relevant to clinicians who might consider prescribing TRE to patients who struggle to manage their weight.

Another critical strength of this study is the inclusion of a control group. We worked with a clinical psychologist to reduce potential bias between our two treatment groups and treated the control

group (CMT) as similar to the TRE group as possible. This includes similar engagement with the study app, daily eating reminders, and wording to suggest beneficial expected outcomes of the eating plan. While there was statistically significant weight loss in the TRE group, there was no difference between the groups. This indicates that participation in a weight loss study alone (even in the control group) may be sufficient to lead to short-term weight loss. In fact, researchers at the University of Alabama at Birmingham are currently investigating if taking daily weight measurements leads to weight loss in obese subjects (NCT04044794). This study has not been completed yet, but it highlights the fact that study interventions may have inadvertent effects on eating habits and weight loss independent of the treatment plan. These data highlight the importance of proper control groups for dietary intervention studies, and caution should be taken when analyzing data from single-arm studies.

A limitation of this study was the lack of dietary intake information collected from subjects. It is unknown how the TRE prescription altered energy and macronutrient intake. In mice, TRF leads to a compensatory increase in feeding during the eating window so that caloric intake between TRF mice and *ad libitum* mice is equal (5). In humans, the data are less clear. Many human studies examining TRE prescribed caloric intake (14, 34, 48); while these are vital to understand how TRE effects metabolism compared to a calorie-matched diet, these studies do not reveal TRE's effects on caloric intake in a real-world setting. We did not measure calorie intake during the study; however, mathematical modeling of changes in energy intake suggests that calorie intake did not significantly differ between groups in our study. While mathematical modeling of energy intake is not perfect, it may be more accurate than self-reported energy intake (24). The mathematical estimate of energy intake is supported by the absence of change in RMR, modest decrease in TEE, and modest weight reduction. Additionally, we did not measure changes in

protein intake. Given the loss of lean mass in our TRE subjects and previous reports of decreased protein consumption from TRE (12, 47), it is possible that protein intake was altered by TRE in this cohort, and this clearly warrants future study. Lastly, the DXA analysis of lean mass did not factor in muscle hydration, so it is possible that changes in hydration could confound our lean mass findings. To help control for this, all subjects fasted for >12 hours and voided their bladder prior to their DXA scans. However, the change in lean mass in the TRE group was -1.10kg, and the change in total body water was only -0.36kg, so even if there were differences in muscle hydration, it could not account for all of the lean mass loss.

4.6 Conclusions

In this randomized controlled trial, a prescription of TRE was not an effective method of weight loss as compared to a control prescription of 3-meals-per-day. While weight did decrease slightly in the TRE group, ~65% of the weight loss was due to losses of lean mass which was associated with a significant reduction in appendicular lean mass. Furthermore, TRE did not influence markers of insulin sensitivity, glucose homeostasis, or CVD risk. Together, the results of this study: 1) call into question the efficacy of TRE for weight loss, 2) warrant caution in prescribing TRE to subjects susceptible to decreased lean mass, and 3) highlight the importance of proper control diets in dietary interventions. Future studies should be aimed at understanding the effects of early versus late TRE and should also focus on protein intake or timing as a means to offset the loss in lean mass.

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