Prolonged Daily Fasting As a Viable Alternative to Caloric Restriction in At-Risk Obese Humans

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STATISTICAL ANALYSIS

The primary hypothesis was that the TRE and CR groups would have similar weight loss relative to baseline, which would be greater than weight loss achieved in the UE group. The study was powered to detect differences in weight loss (randomization to 12 weeks) for TRE and CR relative to UE, while preserving type-1 error for pairwise comparisons across all three groups. Based on the preliminary data and the literature [35] we anticipated weight loss of 3.6 kg (1.9) [mean (SD)] with TRE, 4 kg (2.5) with CR, and 1.5 kg (2.4) with UE. With n=24 per group and alpha = 0.05/3 to adjust for multiple comparisons, we estimated 80% power to detect the described difference in weight loss between TRE vs UE and 85% power to detect the described difference in weight loss between CR group vs UE.

Data were analyzed by intention-to-treat. Pre-post intervention change in outcomes were compared between the three groups using linear regression models with pairwise comparisons conducted using Tukey's method to account for multiple comparisons. The primary efficacy analysis included all randomized individuals with missing outcome measures imputed using multivariate imputation by chained equations. As a sensitivity analysis, the primary outcome analysis was conducted using only individuals who had complete data for the outcome of interest. (Supplementary Table 1). An additional sensitivity analysis among study completers was conducted excluding one participant in the UE group who reported noncompliance and had extreme weight loss (14.1 kg).

Multivariate imputation by chained equations was conducted using predictive mean matching to impute the primary and secondary outcome variables (weight, whole body percent fat, visceral fat, lean mass, fat mass, calorie intake; M-Value from clamp low dose infusion, M-Value from clamp high dose infusion, metabolic flexibility at end of low-dose clamp, metabolic flexibility at end of high-dose clamp and resting energy expenditure. Auxiliary variables included in the imputation included study arm, days from randomization to body composition and clamp assessments, age, sex, and baseline systolic and diastolic blood pressure, fasting : LDL, HDL, triglycerides glucose, insulin, hemoglobin A1C, eating window and healthy eating index. For the imputation of cardiometabolic measures (resting energy expenditure, HDL, triglycerides, LDL, HbA1c, HOMA-IR, fasting glucose, fasting insulin, and CGM measures) auxiliary variables included in the imputation

were study arm, days from randomization to study assessments, sex, age, baseline systolic and diastolic blood

pressure, and baseline weight. For the imputation of dietary and lifestyle measures (calories, HEI, servings of sugar sweetened beverages, and actigraphy measures of activity and sleep) auxiliary variables included study arm, days from randomization to study assessments, sex, age, baseline systolic and diastolic blood pressure, and baseline weight, eating window, fasting glucose, fasting insulin, HDL, triglycerides, and LDL, Linear model results for change in outcome were pooled across 50 imputations using Rubin's rules [36] for computing the total variance.

For representing change in measures using forest plots, outcomes were transformed into z-scores by subtracting the overall mean and dividing by the overall standard deviation prior to fitting the unadjusted linear regression model**.**

The association between change in eating window and pre-post intervention change in outcome among study completers in the TRE and UE groups was assessed using linear regression models. Eating window calculated using time frame encompassing 95% of eating occasions and change in eating window was calculated as difference in eating window from baseline (prior to randomization) and end-of-intervention (Weeks 10-12). The CR arm was not included in these analyses since they were not instructed to document all eating occasions for the duration of the study using the mCC app.

All analyses were carried out using R (Version 4.3.1, R Foundation for Statistical Computing, Vienna, Austria) or SAS (Version 9.4, SAS Institute Inc., Cary, NC).

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