Statistical Analysis Plan for the manuscript reporting the primary outcome in the RESET study

Contents

Section 1, item 1-6: Administrative information

Title: ***Effect of time-restricted eating on behaviour and metabolism in overweight individuals at high risk of type 2 diabetes – the RESET study***

Publication date: 28 February 2021
SAP version: 1
Protocol version: 7
ClinicalTrials.gov Identifier: NCT03854656
Study ID/Grant number: NNF17OC0027822

This document is a supplement to the RESET study protocol and contains the statistical analysis plan (SAP) for the main paper of the trial in which the primary outcome will be reported. This document complies with the guidelines for content of statistical analysis plans in clinical trials (1). The SAP does not cover the statistical analysis of data for outcomes to be included in secondary manuscripts.

Principal investigator Kristine Færch, MSc PhD
Steno Diabetes Center Copenhagen
Borgmester Ib Juuls Vej 83
DK-2730 Herlev
Denmark
E-mail: kristine.faerch@regionh.dk
Phone: +45 3091 3061.

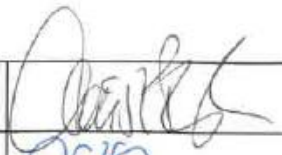
Signature page

To be signed by persons writing the Statistical Analysis Plan (SAP), senior statistician responsible, contributors to the SAP, principal investigator and co-investigators.

Title: ***Effect of time-restricted eating on behaviour and metabolism in overweight individuals at high risk of type 2 diabetes – the RESET study***

ClinicalTrials.gov Identifier: NCT03854656

Name	Title	Role	Signature	Date
Kristine Færch[1,2]	MSc, PhD	Sponsor/Principal investigator, contributor to SAP		22/2-2022
Jonas Salling Quist[1,3]	MSc, PhD	Co-investigator, contributor to SAP		28/2-2022
Marit Eika Jørgensen[1,4,5]	MD, PhD	Co-investigator		22/2-2022
Hanne Pedersen[1,6]	MSc	Co-investigator, contributor to SAP		23/2-2022
Kim Katrine Bjerring Clemmensen[1,7]	MD, PhD	Co-investigator, contributor to SAP		23/2-2022
Marie Møller Jensen[1,8]	Msc	Co-investigator, contributor to SAP		
Martin Bæk Blond[1]	MD, PhD	Dataanalyst, person writing the SAP		23-02-2022
Dorte Vistisen[1,9]	Msc, PhD	Senior Statistician Responsible, contributor to SAP		22/2-2022
Natasja Bjerre[1]	MSc	Co-investigator		22/2-2022
Nicolai Jacob Wewer Albrechtsen[2,10,11,12]	MD, PhD	Co-investigator	Nicolai Albrechtsen	28/2-2022
Jens Juul Holst[2,12]	MD, DMSc	Co-investigator		25/2-22
Signe Sørensen Torekov[2,12]	MSc, PhD	Co-investigator		25/02-22
Satchidananda Panda[13]	MSc, PhD	Co-investigator	Satchidananda Panda	25/02/2022
Graham Finlayson[1,3]	BSc, PhD	Co-investigator		22/2-2022

Christina Brock[8,14,15]	DVMD, PhD	Co-investigator		25/2- 2022
Joachim Størling[1,2]	MSc, PhD	Co-investigator		22.02.2022

Affiliations

1. Steno Diabetes Center Copenhagen, Herlev, Denmark
2. Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
3. School of Psychology, Faculty of Medicine & Health, University of Leeds, Leeds, UK
4. National Institute of Public Health, University of Southern Denmark, Copenhagen, Denmark
5. Steno Diabetes Center Greenland, Nuuk, Greenland
6. iMotions A/S, Frederiksberg, Denmark
7. Novo Nordisk A/S, Søborg, Denmark
8. Department of Clinical Medicine, Aalborg University, Aalborg University, Aalborg, Denmark
9. Department of Public Health, University of Copenhagen, Copenhagen, Denmark
10. Department of Clinical Biochemistry, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
11. Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
12. Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
13. Salk Institute of Biological Studies, La Jolla, CA, USA
14. Mech-Sense, Department of Gastroenterology and Hepatology, Aalborg University Hospital and Clinical Institute Aalborg University, Aalborg, Denmark
15. Steno Diabetes Center North Jutland, Aalborg, Denmark

Section 2: Introduction

7 Background and Rationale (adapted from study protocol)

Animal studies and a few human studies have reported promising effects of alterations in dietary regimens, including intermittent fasting and time-restricted eating (TRE), without concomitant dietary restrictions, on body weight and other cardiometabolic risk factors (2,3). Data suggest that intermittent fasting, e.g. alternate day fasting, may promote modest weight loss and improve glucose homeostasis; however, adherence to the intervention is challenging (4). In contrast, TRE allowing dietary intake within a limited time interval during the day, seems to be a more attractive and feasible option. The TRE regimen allows individuals to consume an *ad libitum* diet within a limited time window, typically ≤12 hours.

8 Objectives

The primary objective of the RESET study is to investigate effects of 12 weeks of TRE (10 hours/day) on change in body weight in individuals at high risk of type 2 diabetes. Secondary objectives are to describe changes associated with the intervention for body composition, metabolism, and behaviour and to assess aspects related to motivation, feasibility, and maintenance.

<u>Hypotheses hierarchy for the primary outcome</u>

1. We hypothesize that TRE is superior to no intervention (the control group) in reducing total body weight from baseline to end-of-treatment (from V1 to V3). Superiority is claimed if:
    a. the 95% confidence interval for the estimated difference in change between the groups for total body weight, estimated using a baseline corrected linear mixed model (see Section 6.27), excludes 0 and the P-value is < 0.05; and
    b. the estimated difference in total body weight between the two groups is equal to/surpass the minimal important difference in favor of the TRE group.
2. We hypothesize that TRE is superior to no intervention (the control group) in reducing total body weight from baseline to follow-up (from V1 to V4). Superiority is claimed if:
    a. the 95% confidence interval for the estimated difference in change between the groups for total body weight, estimated using a baseline corrected linear mixed model (see Section 6.27), excludes 0 and the P-value is < 0.05; and
    b. the estimated difference in total body weight between the two groups is equal to/surpass the minimal important difference in favor of the TRE group.

Hypotheses for descriptive/exploratory outcomes can be found in the study protocol.

Section 3: Study Methods

9 Trial design

Single-centre parallel-group, randomized controlled, open-label, superiority trial. Allocation ratio 1:1 to either TRE or control (no-intervention).

10 Randomization

Participants will be randomized using block randomization with varying block sizes and no stratification for baseline variables, block sizes are unknown to the researchers. The randomization list was generated by an external statistician and uploaded to the electronic data management system REDCap (8.10.18, Vanderbilt University, TN, USA). At V1 the participant will receive a bag secured by a combination lock in which the name of the allocated group is stored. After the free-living assessment period at V1 the participant will be contacted by telephone by the research team and informed about the combination to the lock on the bag. Thereby, the participant will be informed of which group they have been randomised to and will receive instructions accordingly. A randomisation list will be kept securely at the study site by one of the investigators with clinical responsibility.

11 Sample size

See study protocol.

12 Framework

Superity trial. See point 8.

13 Statistical interim analyses and stopping guidance

No interim analyses are planned and no guidelines for terminating the trial early has been made.

14 Timing of final analyses

Results will be analyzed when the last participant completes V4.

15 Timing of outcome assessments

Body weight is measured at the screening (V0), at the baseline test (V1, 0 weeks), after 6 weeks of intervention (V2), after 12 weeks of intervention (V3) and after the follow-up period (V4, 26 weeks)
See item 26 for timing of secondary and descriptive/explorative outcome assessments.

Section 4: Statistical Principles

16-18 Confidence intervals and P values

Two-sided P-values and 95% confidence intervals will be presented for comparisons (between and within groups). Likewise, two-sided 95% confidence intervals will be presented for estimated levels.

Primary outcome: A hierarchal testing procedure will be used to control the type 1 error rate for tests (see item 8); if a test fails to confirm a given hypothesis all subsequent tests within the hierarchi will be regarded as descriptive/explorative. Statistical significance will be claimed if the null hypothesis is rejected at the alpha level of 0.05 (two-sided), i.e. the P-value of the null hypothesis test is < 0.05. The direction and size of the estimated mean effect for total body weight (primary outcome), in addition to the 95% confidence intervals, will be required to support the tested hypothesis in order for the results to be declared in accordance with the hypothesis (see item 8).

Secondary outcomes: False detection rate (FDR) correction ad modem Benjamini and Hochberg (5) will be used to control for multiplicity; < 5% will be used as the threshold for FDR.

Descriptive/explorative outcome assessments: No correction for multiplicity is planned and non-hypothesis based tests are per definition of a descriptive/exploratory nature from which no definite inferences can be made.

19 Adherence and protocol deviations

Compliance:
%-compliance = (number of days compliant / total days) * 100%.
Compliant days are defined as:

- not exceeding the 10-hour eating duration by more than 1 hour daily (i.e., have an eating duration < 11 hours/day); and
- starting less than one hour before and ending less than one hour after the 10-hour self-selected eating window. Eating window delimited by the self-selected start and end of daily intake of food and beverages (except of water).

Days for which a participant has not recorded eating window and/or duration will be regarded as non-compliant days. Study days and other days where the participants per study design cannot follow the TRE will not be included in the calculation of compliance. No compliance criteria for the participants in the control group have been defined.

Completers: Participants who participate in assessment of primary outcome at V3.

Lost to follow-up: Participants who do not participate in assessment of primary outcome at V3.

Per protocol: Completers in the TRE group are considered *per protocol* if their %-compliance is ≥80%. All completers in the control group are considered per protocol.

Protocol deviators: All completers in the TRE group, who are not considered per protocol. No participants in the control are considered protocol deviators.

Summary data/presentation of data: Adherence to the intervention in the TRE group will be presented as the summary data of percentage of days for which the participants are compliant. Additionally, the average eating duration (hours:minutes) for both groups, both during the active intervention period and during the follow-up period. The average volume of time eating outside the self-selected eating window (hours:minutes) for the TRE group will be presented. Data related to measures of adherence will be presented for all completers and for per protocol completers in the TRE group. Intervention duration (days) and % of days where the eating window is registered will be reported for both groups. The distribution of the outcome will be visually inspected using QQ-plots and histograms and if Gaussian distributed it will be presented as means and standard deviations; if not it will be presented as medians plus 25^{th} and 75^{th} percentiles. Difference between groups for average daily eating duration during the intervention and during the follow-up period will be reported.

20 Analysis populations

Intention-to-treat (ITT) analysis set:
All participants will be analyzed as randomized.
Per Protocol (PP) analysis set:
See item 19 for definition of per protocol.

Section 5: Trial Population

21 Screening data

The following data obtained at the screening visit will be included for those that entered the trial:
Self-reported sex
Age
Self-reported ethnicity
Family history of diabetes and cardiovascular disease
Smoking (or other tobacco products)
Medical history
Height

22 Eligibility

- Age: ≥30 to ≤70 years
- Body mass index ≥30 kg/m2 or body mass index ≥25 kg/m2 in combination with pre-diabetes (HbA1c ≥39 to <48 mmol/mol)
- Habitual eating/drinking window ≥12 hours (including foods/snacks and energy containing beverages e.g. soft drinks (except of water)) and an eating/drinking window of ≥14 hours minimum one day per week

23 Recruitment

The flow chart of the trial will comply with the CONSORT guidelines and will include the number of individuals who:

1. received oral information
2. were assessed for eligibility at screening; including the number eligible and the number uneligible
3. consented
4. participated in baseline tests
5. were randomized
6. were allocated to the two groups
7. received the randomized allocation
8. attended each of the visits
9. completed per protocol at V2 and V3
10. were included in the primary analysis

Additionally, the attrition and if possible reasons for attrition will be supplied for each phase of the trial, and if applicable the number of per protocol completers will be given.

24 Withdrawal/follow-up

The level of consent and consent withdrawal will be tabulated (classified as "consent to continue follow-up and data collection", "withdrawal of consent – no further follow-up or data collection"). If a randomized participant wishes to withdraw from the intervention, but is willing to continue in the trial, we will offer to perform all or some of the examinations at V3. Participants without a total body weight measurement at V3 will be regarded as lost-to-follow-up in regards to the main outcome during the intervention period and those with a missing total body weight measurement at V4 will be regarded as lost-to-follow-up in regards to the primary outcome during the follow-up period. The number of participants lost-to-follow-up for each group during each phase of the trial will be reported in the CONSORT diagram (See item 23). Summary of baseline levels for variables reported in the baseline table will be provided for completers and for those lost to follow-up. Spaghetti plots will be used to visualize levels of the main outcome for completers and non-completers.

25 Baseline participant characteristics

The distribution of all continuous outcomes included in baseline characteristics will be visually inspected using QQ-plots and histograms; those with a Gaussian distribution will be presented as means and standard deviations and those with a non-Gaussian distribution will be presented as medians plus 25^{th} and 75^{th} percentiles, number of observations will be presented for each outcome presented. Categorical data will be summarised by numbers and percentages. Tests of statistical significance will not be undertaken for baseline characteristics; rather the clinical importance of any imbalance will be noted.

The following outcomes will be included in the baseline participant characteristics table for all participants combined and stratified by randomization group:

- Number of participants (men/women)
- Age (years)
- Sex (n and % of male/female/other)

- Self-reported ethnicity
  - White ethnicity (n and %),
  - Non-white ethnicity (n and %))
- Current smoker, n (%)
- Education
  - Elementary school (n and %)
  - Education (not University) (n and %)
  - University (n and %)
- Occupation
  - Retired (n and %)
  - Unemployed (n and %)
  - Employed/self-employed (n and %)
  - Other (n and %)
- Living alone, n (%)
- Children living at home, n (%)
- Family history of diabetes, n (%)
- Family history of CVD, n (%)
- Antihypertensive medication, n (%)
- Lipid-lowering medication, n (%)
- Antiplatelet agents/DOACs, n (%)

- Weight (kg)
- BMI (kg/m^2)
- Waist circumference(cm)
- Waist-hip ratio (unitless)
- Total fat mass (kg)
- Total fat free mass (kg)
- Body fat percentage (%)
- A/G-ratio

- Resting energy expenditure (kcal/day)
- Resting substrate oxidation (RER)

- Average daily eating duration (hours:minutes)
- Total energy intake (KJ/day and/or kcal/day)
- Macronutrient intake
  - Carbohydrate (KJ/day and percentage of total energy intake (E%))
  - Fat (KJ/day and E%)
  - Protein (KJ/day and E%)
  - Alcohol (KJ/day and E%)

- Systolic blood pressure (mmHg)
- Diastolic blood pressure (mmHg)

- HbA1c (mmol/mol and %)
- Fasting plasma glucose (mmol/L)
- Fasting insulin (pmol/L)

- Average glucose measured with CGM (mM)
- Glycemic variability
  - CV (%)
  - SD (mmol/L))
- Time in ranges (minutes/day)
  - Time spent at >6.1 mmol/l
  - Time spent at >7.0 mmol/l
  - Time spent at >7.8 mmol/l
  - Time spent at >11.0 mmol/l
- Average of meal test glucose AUC (mmol/L*min)
- Average of meal test insulin AUC (pmol/L*min)

- Fasting concentration of total cholesterol (mmol/L)
- Fasting concentration of LDL cholesterol (mmol/L)
- Fasting concentration of HDL cholesterol (mmol/L)
- Fasting concentration of triglycerides (mmol/L)

- Pittsburgh Sleep Quality Index (PSQI)
  - Global score (0-21)
  - Bedtime
  - Wake-up
  - Sleep duration
- Short Form-36 (SF-36):
  - General health perception
  - Physical functioning
  - Emotional well-being

Section 6: Analysis

26 Outcome definitions

Primary outcome: Total body weight (measured to the nearest 0.1 kg). The treatment effect will be given as the baseline corrected difference in kg between the groups at V3 and V4 (see item 27 for details). The relative difference (%) will also be provided.

Estimation of minimal important difference for primary outcome: There is strong evidence for the clinical relevance of a weight reduction of 3% in people with overweight or obesity, with or without prediabetes (6) and hence a 3% difference in change in bodyweight has been defined as the minimal important difference

for this trial. For the participants with a BMI of 25 kg/m^2 (with an expected mean height of 170 cm) a change in weight of 3% will correspond to ~2 kg. Thus, in order to detect a difference in weight change (3%) across the allowed BMI range, the trial was dimensioned to detect a difference in change of 2 kg between the intervention and control group from baseline to V3. The minimal clinically relevant effect size was consequently defined as being 2 kg of body weight.

Secondary outcomes:

The treatment effect will be given as the baseline corrected difference between the groups at V3 and V4 (see item 27 for details). No minimal clinically relevant differences were defined for these outcomes.

- Total body fat mass (kg)
- Total fat free mass (kg)
- Total energy intake (KJ/day) (not measured at V4)
- HbA1c (mmol/mol and %)
- Fasting plasma glucose (mmol/L)
- Fasting concentration of LDL cholesterol (mmol/L)

Descriptive/explorative outcomes:

Baseline corrected difference at V2 for:

- Total body weight (kg)
- Total body fat mass (kg)
- Total fat free mass (kg)
- Total energy intake (kJ/day)
- HbA1c (mmol/mol and %)
- Fasting plasma glucose (mmol/L)
- Fasting concentration of LDL cholesterol (mmol/L)

Baseline corrected differences at V3 for:

- Average of meal test glucose AUC (mmol/L*min)
- Average of meal test insulin AUC (pmol/L*min)
- Resting energy expenditure (kcal/day)
- Resting substrate oxidation (RER)

Baseline corrected differences at V2 and V3 for:

- Macronutrient intake
  - Carbohydrate (KJ/day and percentage of total energy intake (E%))
  - Fat (KJ/day and E%)
  - Protein (KJ/day and E%)
  - Alcohol (KJ/day and E%)
- Average glucose measured with CGM (mM)
- Glycemic variability

  - CV (%)
  - SD (mmol/L))
- Time in ranges (minutes/day)
  - Time spent at >6.1 mmol/l
  - Time spent at >7.0 mmol/l
  - Time spent at >7.8 mmol/l
  - Time spent at >11.0 mmol/l

Baseline corrected differences at V2, V3, and V4 for:

- BMI (kg/m^2)
- Waist circumference(cm)
- Waist-hip ratio (unitless)
- Body fat percentage (%)
- A/G-ratio
- Systolic blood pressure (mmHg)
- Diastolic blood pressure (mmHg)
- Fasting insulin (pmol/L)
- Fasting concentration of total cholesterol (mmol/L)
- Fasting concentration of HDL cholesterol (mmol/L)
- Fasting concentration of triglycerides (mmol/L)
- PSQI:
  - Global score (0-21)
  - Bedtime
  - Wake-up
  - Sleep duration
- SF-36:
  - General health perception
  - Physical functioning
  - Emotional well-being

Other

- Number and percentage of participants losing >=3% and >=5% of total body weight from V1 to V3 and from V1 to V4.

27 Analysis methods

Analyses of the primary outcome (except for the pre-planned per protocol) will be performed based on the intention-to-treat (ITT) principle.

Before further analysis and before unblinding, all variables will be inspected to detect outliers in order to uncover potential errors, such as registration errors. Data for all participants with a change from baseline of more than 5% of total body weight will checked to rule out registration error. In the case of evidence of a registration error, this will be corrected, otherwise the value will remain unchanged.

All continuous endpoints/outcomes covered by this SAP will as a rule be modelled using baseline corrected repeated measures regression (7) with the following fixed effects and interactions between fixed effects: Treatment, Visit (factorial)*Treatment. Data from V1, V2 (for those outcomes measured at V2), V3 and V4 (for those outcomes measured at V4) will be included in the analysis. All participants will be placed in the control group at V1 in the statistical analysis. The models will be specified with a restricted maximum likelihood estimation method and a repeat on participant level (unstructured covariance structure). Model fit will be evaluated using graphical methods before estimating the treatment effects and if necessary, outcomes will be log-transformed. Estimated mean differences (CI95%) between groups (CI95%), conditional means (CI95%), and within group changes (CI95%) will be extracted from the model. For log-transformed outcomes the results will be back-transformed and be presented as the ratio between estimated mean differences (CI95%), estimated conditional geometric means (CI95%) and relative changes within groups (CI95%), respectively. If distribution assumptions cannot be met by log-transformation, a generalized mixed linear model with an appropriate distribution will be applied instead of the repeated measures regression model. In case the distribution does not comply with the distributions availabe in the generalized mixed linear model a non-parametric test will be used to compare the change scores for the given outcome.

Between group differences will be null-hypothesis tested and presented with P-values.

The following two suplementary analyses are planned for the primary outcome:

1. An analysis similar to the one described above, but including the per-protocol analysis population.
2. A sensitivity analysis, including the ITT analysis population, assessing the impact of missing data for the primary outcome. This analysis will only be performed in the case of a loss-to-follow-up rate > 10%, ie more than 10% of all participants are missing at V3 and/or V4 (see also item 28).

The analysis of the primary and secondary outcomes will be performed blinded to group allocation by a researcher that have not been involved in the execution of the trial.

28 Missing data

The number/frequency of missing values for the primary outcome in each group at each time point will be provided. In the primary analysis, missing data are handled implicitly by maximum likelihood estimation in the linear mixed model and missing data will be assumed to be missing at random. This is in the model specified in point 27 equivalent to making multiple imputations for each treatment group separately and estimates the treatment effect that would have been found had all subject completed their assigned treatment (efficacy estimate) under the missing at random assumption. In the case that >10% of the participants are lost-to-follow-up we will perform a supplementary analysis to challenge the assumptions of “missing at random” in the primary analysis. We will assume that the development in body weight among the participants from TRE that are lost-to-follow-up will resemble the development in the control group rather than the development in the group to which they were originally randomized. Participants from the TRE group with missing values at V3 and/or V4 will be pooled with the control group and using a Markov chain Monte Carlo method all missing values will be imputed to create 1000 new datasets, assuming a multivariate normal distribution for the data. The dataset used for the imputation will include all previous measurements for weight (excluding that from the screening visit), age and sex. Subsequently, the imputed

datasets will be analyzed using a mixed linear model including the same variables included in the main analysis and averaged estimates will be calculated. If the main outcome has been transformed to fit the statistical model used for the main analysis this transformation will be applied to the outcome prior to the imputation procedure.

29 Additional analyses

Not relevant

30 Harms

Data on harms are not systematically collected and will not be reported.

31 Statistical software

R version 3.6.0 or newer version (The R Foundation for Statistical Computing, www.R-project.org) and SAS version 9.4 or newer version (SAS Institute, Cary, NC, USA).

References

1 Gamble C, Krishan A, Stocken D, Lewis S, Juszczak E, Doré C *et al.* Guidelines for the content of statistical analysis plans in clinical trials. *JAMA - J Am Med Assoc* 2017; **318**: 2337–2343.

2 Mattson MP, Allison DB, Fontana L, Harvie M, Longo VD, Malaisse WJ *et al.* Meal frequency and timing in health and disease. *Proc Natl Acad Sci* 2014; **111**: 16647–16653.

3 Chaix A, Zarrinpar A, Miu P, Panda S. Time-restricted feeding is a preventative and therapeutic intervention against diverse nutritional challenges. *Cell Metab* 2014; **20**: 991–1005.

4 Patterson RE, Sears DD. Metabolic Effects of Intermittent Fasting. *Annu Rev Nutr* 2017; **37**: 371–393.

5 Benjamini Y, Hochberg Y. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B* 1995; **57**: 289–300.

6 Jensen MD, Ryan DH, Apovian CM, Ard JD, Comuzzie AG, Donato KA *et al.* 2013 AHA/ACC/TOS Guideline for the Management of Overweight and Obesity in Adults. *Circulation* 2014; **129**: S102–S138.

7 Liu GF, Lu K, Mogg R, Mallick M, Mehrotra D V. Should baseline be a covariate or dependent variable in analyses of change from baseline in clinical trials? *Stat Med* 2009; **28**: 2509–2530.