STATISTICAL ANALYSIS PLAN

Study Title:

Cut Down on Carbohydrate in the Dietary Therapy of Type 2 Diabetes - The Meal Box Study

Protocol Acronym: CutDM-MealBox

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Trial Statistician: Brice Ozenne, post.doc in biostatistics, University of Copenhagen

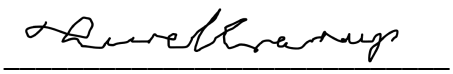
Version History

Version	Date	Description of Changes	Authors
1.0	05-02-2026	Final Statistical Analysis Plan	Rasmus M Bastkjær, Luise Kopp-Nilsson

Signatures

The undersigned hereby confirm that the Statistical Analysis Plan v. 1.0 has been reviewed and approved.

Principal Investigator: Thure Krarup Date: 09-02-2026

Signature: 

Trial Statistician: Brice Ozenne Date: 06-02-2026

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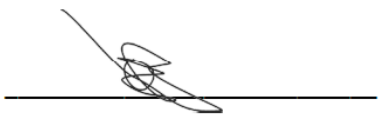

Table of content

SAP

Derived from the pre-specified statistical methods described in the published study protocol.

1. General framework

The general framework applies to all outcomes unless stated otherwise.

The intention-to-treat analysis will be conducted to estimate the effect of prescribing/encouraging a CRHP eating pattern and may therefore differ from the effect of the eating pattern in the presence of imperfect adherence to the diet by the participant.

Primary outcome: HbA1c

Secondary outcomes: Weight and Intrahepatic fat content

Covariate adjustment (value at baseline):

- Sex
- Age
- Weight in kg
- Type 2 diabetes duration in years
- HOMA2-IR index (Homeostatic Model Assessment 2 for Insulin Resistance)
- Glucose-lowering medication at baseline, quantified as the number of glucose-lowering medication classes used (0–4): metformin, DPP-4 inhibitors, SGLT-2 inhibitors, and GLP-1 receptor agonists.

1.1. Analysis sets

Definition of participants included in the analyses is described in the Full Analysis Set (FAS) and Per Protocol Set (PPS).

Intercurrent events leading to study discontinuation: participants who permanently discontinued study participation before the planned end of study are described with respect to timing and reason for premature discontinuation in section 2.

1.1.1. Full Analysis Set

The Full Analysis Set (FAS) follows the Intention-to-treat (ITT) principle and includes all randomized participants, except where explicitly stated otherwise.

<u>Exclusion of randomized participants from the FAS:</u>

One randomized participant did not meet an eligibility criterion (presence of cancer) that was assessed by MRI at baseline, prior to randomization. The MRI result became available two weeks after initiation of the intervention, at which point the eligibility violation was identified.

Consequently, this participant will be excluded from the FAS, and all data from this participant, including baseline data, will be excluded from the analyses.

1.1.2. Per Protocol Set

Purpose: PP analyses will be conducted as supportive analyses to complement the primary intention-to-treat (ITT) analyses (1).

The different PPS of subjects and analysis are described in section 6.

1.2. Missing values due to intercurrent events

For the primary and secondary analyses, missing values will be handled under the hypothetical strategy assuming that missing values follow the same distribution as the observed data, conditional on the model assumptions. This strategy will be carried out by constrained linear mixed models (cLMM) using a full information approach as recommended by regulatory guidelines (2,3).

1.3. Estimation, Confidence Intervals and Hypothesis Testing

Treatment effect will be reported as an estimated marginal mean, with two-sided 95% confidence intervals (CI) and p-values, and will be considered statistically significant if the 95% CI does not include 0.

Multiplicity control and role in inference
Outcomes are ranked *a priori* according to clinical importance as follows:
(1) Primary outcome: change in HbA1c;
(2) Secondary outcome 1: change in body weight;
(3) Secondary outcome 2: change in intrahepatic fat content.

All three analyses will be carried out without adjustment for multiplicity.

The two subgroup analyses of the secondary outcome 2 (intrahepatic fat content) – MASLD-status at baseline and elevated intrahepatic fat content at baseline - will be conducted only if evidence of a treatment effect is observed for the overall intrahepatic fat content outcome. If this condition is not met, subgroup analyses will be reported descriptively, with estimates and standard errors presented; any p-values or confidence intervals will be considered exploratory and hypothesis-generating.

Superiority and non-inferiority testing:
Non-inferiority should be tested first and if rejected superiority should then be tested. However since demonstrating superiority implies non-inferiority and is more readily available in software, superiority will be tested first.

If superiority testing fails to reject the null hypothesis, non-inferiority testing will be conducted secondarily for the primary and secondary outcomes (4):

- using a non-inferiority margin of 3 mmol/mol for change in HbA1c, and
- a non-inferiority margin of 5% for weight loss, and
- a non-inferiority margin of 25% relative change in intrahepatic fat.

Switching from superiority to non-inferiority testing will be performed without adjustment for multiple testing, in accordance with regulatory guidance (4).

1.4. Execution of the statistical analyses

The statistical analyses will be conducted by Rasmus Bastkjær Mainz Hansen and Luise Helene Winther Kopp-Nilsson with assistance from Brice Ozenne.

2. Handling of data: not-existing, not-evaluable or not available

2.1. Intercurrent events leading to discontinuation

The following intercurrent events lead to premature study discontinuation and incomplete data records, resulting in not-existing outcome data at different time-points:

- Inability to complete the intervention due to personal or logistical reasons, such as deployment, time constraints, or relocation away from the study site.
- Initiation of prohibited medication according to the study protocol such as sulfonylureas, or systemic glucocorticoids.
- Occurrence of serious non-treatment-related intercurrent illnesses, such as stroke.
- Withdrawal from the study prior to initiation of the intervention due to dislike of the intervention.
- Discontinuation of the intervention due to dislike of the food/diet.

Collection of outcome data at time points subsequent to the above-mentioned intercurrent event was not possible and does therefore not exist for participants with intercurrent events that lead to study discontinuation. Reasons for and timing of study discontinuation are described in Table 1.

Table 1) Intercurrent events leading to discontinuation or withdrawal (10/100 participants)

Rand. No.	Reason	Date	Intervention started?	Intervention duration	Last study visit
11	Treatment with rescue medication (sulfonylurea) (exclusion criteria)	19/5/2023	yes	3 months	3-month
13	Stroke	10/5/2023	yes	3 months	Baseline
19	Time constraint to fulfill intervention	15/1/2023	yes	5 weeks	Baseline
23	Deployment	01/07/2023	yes	7 months	6-month
25	Severe protocol deviation (exclusion criteria)	12/6/2023	yes	6 months	6-month
26	Treatment with rescue medication (Sulfonylurea) (exclusion criteria)	10/1/2023	yes	3 weeks	Baseline
28	Treatment with glucocorticoids (exclusion criteria)	17/01/2023	yes	42 weeks	6-month
38	Disliked food/diet	7/3/2023	yes	3 weeks	Baseline
39	Disliked intervention	24/2/2023	no	-	Baseline
50	Relocation far away from the study site	20/2/2024	yes	10 months	9-month

2.2. Intercurrent events affecting existence or interpretability of specific outcome measurements

Non-evaluable data for the primary and secondary outcomes that could not be validly obtained or interpreted due to intercurrent events, including missing study visit, insufficient sample quality, technical failure, or non-compliance of study procedures, is described in the following for the different outcome at the different time-points.

HbA1c

Not evaluable due to insufficient sample quality or loss of sample:

- Month-9: rand. no. 33

- Month-12: rand no. 37

Missing study visit:

- Month-9: rand. rand no. 37

Weight

Missing study visit:

- Month-9: rand. rand no. 37

MRI-derived outcomes not-evaluable or not available

MRI measurements were discontinued due to participant non-compliance (e.g., claustrophobia):

No MRI data available at baseline and month-12:

- Participants rand. no. 24, rand. no. 47

No MRI data available at month-12:

- Participant rand. no. 27

MRI assessments were not obtained due to technical or operational constraints:

No MRI data available at month-12:

- Participants rand. no. 25, rand. no. 90

Incomplete MRI data at baseline (specific compartments):

- MRI data not obtained for visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT), psoas fat and volume, and pancreatic fat content: participant rand. no. 83
- MRI data not obtained for psoas volume: participant rand. no. 81
- MRI data not obtained for VAT: participant rand. no. 14

Incomplete MRI data at baseline and month-12 (specific compartments):

- MRI data not obtained for VAT, psoas fat and volume, and pancreatic fat content: participant rand. no. 94

Incomplete MRI data at month-12:

- MRI data not obtained for psoas fat and volume and pancreatic fat content: participants rand. no. 56, rand. no. 93
- MRI data not obtained for psoas fat and volume only: participant rand. no. 75
- MRI data not obtained for VAT, SAT, psoas fat and volume, and pancreatic fat content: participant rand. no. 69

3. Primary outcome: HbA1c

Estimand: In individuals with type 2 diabetes, what is the mean between-group difference in change in HbA1c (mmol/mol) from baseline to month 12 between those prescribed a carbohydrate-reduced high-protein (CRHP) diet and those prescribed a conventional diabetes (CD) diet, assessed in the intention-to-treat population.

Outcome: change in HbA1c (mmol/mol) in original form from baseline to 12 months.

Model: cLMM using HbA1c measured at baseline, 3 months, 9 months, and 12 months.

Covariate adjustment (pre-specified): adjusting for covariates as specified in the general framework.

Mean structure: 15 mean parameters

- one at baseline,
- one for each group (2 x) at each follow-up time point (4 x (3, 6, 9, 12 months))

One for each of the baseline covariates (sex, age, weight, type 2 diabetes, HOMA2-IR, and glucose-lowering medication) assuming a linear effect on the mean for the continuous covariates and no interactions.

Within-subject covariance:

- unstructured residual covariance matrix
- stratified by the treatment group

To fit this model, the lmm() function from the R package LMMstar (version 1.1.0) will be used, which implements repeated measurement models for discrete time points with flexible covariance structures and statistical inference based on Satterthwaite degrees of freedom approximations (5). In the case where the optimizer returns warnings indicating optimization issues, alternative optimizers will be used instead (e.g., mmrm() from the R package mmrm (Mixed Models for Repeated Measures, version 0.3.x))(6). If this is unsuccessful, a simpler covariance pattern will be considered (Toeplitz stratified on only treatment arm, unstructured covariance without stratification).

Primary contrast/test (hypothesis test):

- Estimated marginal mean difference in changes from baseline to 12 months between groups
- Tested with a Wald test on the relevant cLMM parameter (interaction group × 12 months). Standard error derived from the observed information matrix and degree of freedom evaluated using Satterthwaite approximation (default in LMMstar)(5). A similar strategy would be used with mmrm() but based on the package default: expected information and Kenward-Roger degrees of freedom.

Superiority and non-inferiority:

- Superiority will be tested first (to reject the null hypothesis).
- If superiority is not demonstrated, non-inferiority will be assessed using a non-inferiority margin of 3 mmol/mol for change in HbA1c**.**

Handling of missing values: handled in the cLMM via a full information approach.

Sensitivity analyses:
Sensitivity analyses will be performed to assess the robustness of the primary analysis. These will include evaluation of potential non-linear covariate effects. In addition, the impact of missing values will be explored using a conservative missing-values scenario, more conservative than the cLMM assumption. Specifically, missing values will be imputed assuming outcomes that are two standard deviations worse than those observed, representing a worst-case scenario for participants who discontinued the study.

4. Secondary outcomes:

4.1. Body weight

Estimand: In individuals with type 2 diabetes, what is the mean between-group difference in percentage change in body weight from baseline to month 12 between those prescribed a CRHP diet and those prescribed a CD diet, assessed in the intention-to-treat population.

Outcome: Change in body weight expressed as percentage change from baseline to 12 months.

Model: cLMM using body weight measured at baseline, 3 months, 9 months, and 12 months.

Covariate adjustment: adjusting for covariates as specified in the primary outcome framework.

Mean structure: 15 mean parameters (identical to the primary outcome)

Superiority and non-inferiority:

- Superiority will be tested first.
- If superiority is not demonstrated, non-inferiority will be assessed using a non-inferiority margin of 5 percentage points in percentage weight loss.”

4.2. Intrahepatic fat content

Estimand: In individuals with type 2 diabetes, what is the mean between-group difference in change in intrahepatic fat content from baseline to month 12 between those prescribed a CRHP diet and those prescribed a CD diet, assessed in the intention-to-treat population.

Outcome: Change in log-transformed intrahepatic fat content from baseline to 12 months.

Model: cLMM using log-transformed intrahepatic fat content at baseline and 12 months.

Covariate adjustment: adjusting for covariates as specified in the primary outcome framework.

Mean structure: 9 mean parameters:

- One at baseline and one for each group at follow-up (12 months).
- 6 covariate effects

Superiority and non-inferiority:

- Superiority will be tested first.
- If superiority is not demonstrated, non-inferiority will be assessed using a non-inferiority margin of 25% relative change in intrahepatic fat**.**

4.2.1. Sub-analysis of metabolic dysfunction–associated steatotic liver disease (MASLD) status

A sub-analysis based on intrahepatic fat content will evaluate changes in MASLD status between treatment groups as a binary outcome.

Estimand: In participants with type 2 diabetes and MASLD at baseline, defined as intrahepatic fat content ≥5.0% measured by MRI, the estimand is the between-group difference in the probability of MASLD resolution at 12 months, comparing the CRHP diet with the CD diet, assessed in the ITT population reported as relative risk (RR). MASLD resolution is defined as intrahepatic fat content <5.0% at 12 months.

Analysis population
The sub-analysis population will include participants with intrahepatic fat content ≥5.0% at baseline, as assessed by MRI.

Outcome: MASLD status will be derived from intrahepatic fat content measured by MRI

- MASLD status is defined as intrahepatic fat content ≥5.0%
- Non-MASLD status is defined as intrahepatic fat content <5.0%

The binary outcome is defined as resolution of MASLD at 12 months (yes/no) among participants classified as having MASLD at baseline.

Descriptive analysis
The number and proportion of participants transitioning in MASLD status from baseline to 12 months (MASLD → non-MASLD) will be summarized by treatment group.

Secondary transition analysis
Among participants with MASLD at baseline and 12 months, the probability of MASLD resolution at 12 months will be analyzed using logistic regression, with treatment group as the main

explanatory variable. The analysis will be adjusted for the same baseline covariates as specified for the primary outcome analysis. The statistical significance of the group difference will be based on the p-value of the odds ratio estimated by the logistic regression. The estimand will be estimated by standardization which takes the ratio between (i) the average predicted probability by the logistic regression had all subjects CRHP diet (ii) the average predicted probability by the logistic regression had all subjects CD diet. The standardized risk ratio will be reported in addition to the odds ratio to facilitate clinical interpretation.

4.2.2. Sub-analysis of intrahepatic fat content among participants with elevated baseline intrahepatic fat content

A sub-analysis based on MRI-derived intrahepatic fat content will evaluate changes in intrahepatic fat content over time among participants with elevated liver fat at baseline**,** defined as intrahepatic fat content ≥5.0%.

Estimand: In participants with intrahepatic fat content ≥5.0% at baseline, the estimand is the mean between-group difference in change in intrahepatic fat content from baseline to 12 months**,** comparing the CRHP and CD groups.

Analysis population
The sub-analysis population will include participants with intrahepatic fat content ≥5.0% at baseline, as assessed by MRI.

Outcome: intrahepatic fat content assessed as an continuous variable, measured at baseline and 12 months.

Descriptive analyses
Baseline and 12-month intrahepatic fat content, as well as within-group mean change from baseline to 12 months**,** will be summarized by treatment group using appropriate descriptive statistics.

Secondary analysis
Changes in intrahepatic fat content from baseline to 12 months will be analyzed using a cLMM, with treatment group as the main explanatory variable.
Adjustment for baseline covariates will follow the same baseline covariates as specified for the primary outcome and modelling strategy as specified for the primary outcome analysis.

5. Exploratory outcomes

Outcome types:

- Continuous metabolic outcomes (e.g., lipids)
- Categorical outcomes, where applicable

Analysis methods:

- Continuous outcomes:
- Analyzed using cLMMs, applying a similar general modelling framework as described for the primary outcome, as appropriate for the outcome and available time points
- Categorical outcomes:
- Analyzed using Fisher's exact test for association with treatment group

Role in inference: Exploratory and hypothesis-generating

- No adjustment for multiplicity

6. Per-protocol analyses

The per-protocol (PP) analyses aims to explore the effects on the glucometabolic outcomes under different conditions, such as "acceptable dietary adherence", stable glucose-lowering medication, and completion of 12 months of intervention. In addition, PP analyses will be conducted in a subpopulation defined by baseline C-peptide (connecting peptide) levels, restricted to participants in the upper three quartiles (i.e., excluding the lowest quartile).

Separate PP analyses will be performed to evaluate the effects conditioned on different PP populations as described below.

Role in inference (secondary and supportive):

The primary estimand of interest remains the ITT effect of prescribing and encouraging a CRHP eating pattern. PP analyses will be conducted as supportive analyses to complement the primary ITT analyses and will be interpreted in conjunction with, but not as a replacement for the primary analyses. Results from PP analyses will be considered exploratory and hypothesis-generating, and no adjustment for multiplicity will be applied.

Analysis population

The PP population will include the ITT population and one of the following conditions, as detailed in the separate PP analyses sections:

- acceptable diet adherence to the assigned dietary intervention according to the predefined PP criteria mentioned below (three different definitions of "acceptable diet adherence")
- stable glucose-lowering medication
- completion of 12 months of intervention
- non-low baseline C-peptide levels, as defined by the upper three quartiles

Statistical methods:

PP analyses will be conducted using the same statistical modelling framework as specified for the primary ITT analyses.

- Longitudinal continuous outcomes will be analyzed using cLMMs**.**
- Model structure, covariate adjustment, estimation, and inference will follow the same principles as described for the ITT analyses, unless otherwise stated.

Handling of missing values: No additional imputation beyond the full information approach inherent to the mixed-model framework will be applied.

Interpretation

PP estimates may differ from ITT estimates due to:

- imperfect adherence to the dietary intervention,
- selection effects related to protocol compliance, and
- exclusion of participants with protocol deviations.

Accordingly, PP results will be interpreted with caution and will not be used as the primary basis for inference regarding treatment efficacy.

6.1. PP analyses for dietary adherence

Estimand: In individuals with type 2 diabetes who did not strongly dislike the assigned diet, the estimand is the between-group difference in mean change in HbA1c (mmol/mol), mean change in body weight change (%), and mean change in hepatic fat content (%) from baseline to 12 months, comparing continuous adherence to a CRHP diet versus continuous adherence to a CD diet, under a hypothetical scenario in which all participants remained free of serious intercurrent illness (e.g. stroke), had sufficient time to follow the intervention, had “acceptable dietary adherence”, and did not relocate during the study period.

Handling of intercurrent event

- Disliked diet: principal strata (exclude from analysis)
- Serious disease: hypothetical (censor and impute using cLMM)
- Time constraint or relocation: hypothetical (censor and impute using cLMM)
- Change of medication: hypothetical (censor and impute using cLMM)

Sensitivity analyses
A similar sensitivity analyses to the one described under the primary outcome will be conducted in the PP analyses to impute a “worst case scenario”.

Assessment of dietary adherence

Adherence will be based on:

- food records, and
- carbohydrate intake (no requirements for intake of protein and fat).

"Acceptable dietary adherence" is defined as:

- CRHP diet: 20–40 E% from carbohydrates
- CD diet: 45–65 E% from carbohydrates

6.1.1. PP analyses for dietary adherence based on average intake at later time points

The PP analyses will be conducted to explore treatment effects under conditions of "acceptable dietary adherence". The primary PP definition of dietary adherence is based on average carbohydrate intake at later time points, reflecting the expectation that dietary patterns stabilize over time.

Primary definition of dietary adherence: Participants will be considered adherent if the average carbohydrate intake across the 9- and 12-month visits meets the predefined criteria for the assigned diet. Participants who do not meet the adherence criteria will be censored from the PP analysis from the time point at which non-adherence is identified onward; however, data from earlier visits (e.g. 3 and 6 months) will remain included in the analysis.

Alternative definitions of dietary adherence

As this statistical analysis plan is finalized prior to any analysis of dietary adherence data, the distribution of adherence under the primary definition is unknown. If the primary definition results in very high or very low adherence rates, alternative PP analyses based on predefined adherence definitions will be conducted to assess robustness.

The following alternative definitions may be applied:

Adherence at a minimum of three out of four visits

Participants will be considered adherent if acceptable dietary adherence is observed at a minimum of three out of four scheduled follow-up visits at 3, 6, 9, and 12 months. For participants who fail to meet the adherence criteria, data from the second visit at which non-adherence is identified and all subsequent visits will be censored from the corresponding PP analysis.

Adherence at all scheduled visits

Participants will be considered adherent if acceptable dietary adherence is observed at all scheduled follow-up visits at 3, 6, 9, and 12 months. For participants who do not meet the adherence criteria, data from the first visit at which non-adherence is identified and all subsequent visits will be censored from the corresponding PP analysis.

6.2. PP analyses for medication stability

For the PP analyses of the primary and secondary outcomes, the PP population will include participants who maintained stable background medication throughout the study period.

Stable background medication is defined as no initiation, discontinuation, change in dosage of glucose-lowering medication during the study period.

Application in the PP analyses

- PP analyses of the primary and secondary outcomes will exclude data from the first visit at which unstable glucose-lowering medication is identified and all subsequent visits will be censored from the corresponding PP analysis.

6.3. PP analyses including participants within the upper three quartiles of baseline C-peptide

For the PP (PP) analyses of the primary and secondary outcomes, the PP population will be restricted to participants with baseline C-peptide levels within the upper three quartiles. Participants classified in the lowest quartile of baseline C-peptide will be censored from the PP analysis population.

Application in the PP analyses

- PP analyses of the primary and secondary outcomes will exclude post-baseline data from participants identified as belonging to the lowest quartile of baseline C-peptide levels.
- Baseline data from these participants will remain included, while data from all subsequent follow-up visits will be censored from the corresponding PP analyses.

6.4. PP analyses including participants completing the 12-month intervention

PP analyses of the primary and secondary outcomes, where the PP population will be restricted to participants who completed the full 12-month intervention period.

Application in the PP analyses

- PP analyses of the primary and secondary outcomes will include only participants who completed the 12-month intervention.
- For participants who did not complete the intervention, baseline data will remain included, while data from all post-baseline visits following premature discontinuation will be censored from the corresponding PP analyses.

7. References

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