STATISTICAL ANALYSIS PLAN (SAP)

Low-carbohydrate Versus Low-fat Breakfast in Type 2 Diabetes

Trial Registration: NCT04550468

Version 2: November 17, 2021 with dated adjustments up to June 23, 2022

BACKGROUND AND AIM:

From December 2019 to June 2022, we completed the study titled "Impact of a low-carbohydrate versus low-fat breakfast on blood glucose control in type 2 diabetes" at two study sites, at the University of Wollongong (UOW) and the University of British Columbia (UBC). The aim of the trial was to determine whether a low-carbohydrate high-fat (LC) breakfast (with breakfast meals focused around eggs as a main ingredient), compared to a standard low-fat control (CTL) breakfast (designed to have no/minimal eggs) would improve blood glucose control in individuals with type 2 diabetes (T2D). The primary outcome measure was change in Hemoglobin A1C (HbA1c) following the 12-week dietary intervention. Secondary outcomes included changes in satiety and daily caloric intake, body weight, body mass index, glucose control assessed by continuous glucose monitoring, insulin, C-reactive protein, blood lipid profile, carotenoid levels, and cognitive function. It was hypothesized that consuming a LC breakfast would improve blood glucose control, increase satiety, and improve body composition and cognitive function in people with T2D.

SAMPLE SIZE CONSIDERATIONS

We used a previous breakfast intervention in T2D along with our recently published one-day egg-based low-carbohydrate (LC) breakfast trial to calculate the sample size for this RCT. Rabinovitz et al [1] compared a "big breakfast" containing higher fat and protein providing 33% total daily energy to a small breakfast providing 12.5% daily energy over 3 months in a similar sample of participants with T2D using a similar parallel-group design. They reported an absolute reduction in HbA1c% of 0.46% in the big breakfast group. This is similar to the magnitude of change observed in a recent study by Nichols et al [2] who reported an average HbA1c reduction of 0.45% in response to metformin, the standard prescribed medication for people newly diagnosed with T2D. For this reason, it is reasonable to use the study published by Rabinovitz et al to estimate a clinically relevant effect size for our sample size calculation. A medium effect size (Cohen's d = 0.46) was calculated from the Rabinovitz study. In agreement, for our recent one-day study examining an egg-based LC breakfast (3), the effect size (Cohen's d) for improvements in CGM-derived glucose control variables (including AUC, iAUC, glycemic variability, and mean) ranged from d = 0.48-1.2.

Taking a conservative approach, we used an effect size of d = 0.4 in the sample size calculation. The total sample size necessary to detect a between-within interaction using a repeated measures ANOVA with an effect size of d = 0.4 (which corresponds to an ANOVA effect size f = 0.2) with 90% power and 5% type 1 error with a conservative correlation among repeated measures of r = 0.5 is 68 (n = 34 per group, calculated using G*Power v3.1.9.2). To account for a 20% dropout rate (2), we aimed to recruit a sample size of n = 41 per group. These calculations were documented in the original grant application (Feb 2019), trial protocol (Dec 6, 2019), and SAP (Nov 17, 2021).

Adjustment as of 16th January 2022:
Recruitment was originally closed on November 2021 after n = 92 participants were randomized; however, based on the nature of the remote trial and participants dropping out after randomization, only 78 participants had baseline A1c data. Unexpectedly, 4/35 participants who completed the study in one group failed to obtain follow-up A1c and 2/33 in the other group failed to obtain follow-up A1c, resulting in complete data on n = 31 per group. Reasons included the impact of COVID-19 and winter holidays coinciding with study conclusion or were not reported by the participants. A blinded analysis of the primary outcome by an independent statistician not involved in any other aspect of the trial was completed on January 16, 2022. After consultation with the statistician, the research team made the decision to re-open recruitment in order to obtain sufficient sample size based on the original calculations. Using an updated estimation of loss to follow up (62 complete data out of 92 randomized = 33%), we aimed to recruit an additional 10-12 participants in order to maximize the chances of having n = 34 per group for final analyses.

ANALYSIS SET AND STUDY POPULATIONS

The primary outcome of the study will be analyzed according to the intention-to-treat (ITT) principle. The full analysis set of data will be derived from the set of all randomized participants. Participants allocated to a treatment group (i.e., LC or CTL) will be analyzed as members of that group irrespective of their compliance to the planned treatment. Those with both missing baseline and follow-up value will not be included in the analysis, which is unbiased under a plausible missing at random assumption. All outliers that are within the realm of biological plausibility will be included in the analysis and only obvious data errors (e.g., resulting from technical issues) will be excluded. If there are statistical outliers within the realm of biological plausibility (>2.2*IQR from mean), the data will be presented with and without the outliers included for completeness.

ANALYSIS OBJECTIVES, OUTCOMES, AND STATISTICAL METHODS

All data were collected longitudinally. Statistical analysis will be performed blinded to participant allocation after the last participant has completed the trial.

Primary endpoint: The prespecified primary outcome for this trial will be a comparison of the change in HbA1c (in the standard percentage units) from baseline to 12 weeks between the dietary intervention groups.

The analysis of the primary outcome is based on a superiority approach (i.e., we expect that the LC intervention will be superior in leading to decreased HbA1c compared to the CTL intervention). Data will be analyzed by constrained longitudinal data analysis (cLDA) via a linear mixed model with fixed effects for timepoint (baseline and week 12), the interaction between timepoint and dietary intervention group, and stratified allocation factors (sex) as well as random effects for participants to account for the correlation of repeated measures within participants.

The cLDA approach constrains the baseline means to be equal between the treatment groups, which is a reasonable assumption in the context of a randomized controlled trial. If a participant does not have any HbA1c recordings in the end of the trial, only the baseline value of that participant will be included in the model and no post-intervention value for that participant will be included. No missing data will be imputed as per contemporary guidelines[3]. Model specification will be assessed visually using normal probability plots and residuals vs. fitted values plots. Models will be run using log-transformed outcome variables when departure from model assumptions is observed.

Secondary outcome measures: To supplement the primary outcome of A1c, continuous glucose monitors (CGMs) will be worn by participants during the first and last 14 days of the intervention (i.e., both timepoints of CGM will be collected while participants are consuming their allocated breakfast). Changes in CGM variables (fasting glucose, mean glucose, maximum glucose, minimum glucose, 24-h area under the curve, incremental area under the curve, MAGE, SD, time below 3.9 mmol/L, time above 10mmol/L , time in range (3.9-10 mmol/l); 2-hour post-meal: incremental area under the curve, mean glucose, maximum glucose and SD will be calculated for each 14 day period. CGM data will be analyzed using a linear mixed model that will include fixed effects for timepoint (First 14 days vs Last 14 days), group (LC vs. CTL), their interaction, and stratified allocation factors (sex) and random effects for participants. In this analyses the main effect of group will be most important to identify an overall effect of the intervention on glycemic control variables.

Continuous secondary outcomes will be analyzed similarly to the primary outcome using a constrained baseline longitudinal data analysis linear mixed model (with all relevant follow-up timepoints included as a fixed factor in the model). Secondary outcomes include:

1. Changes in self-reported satiety and fullness assessed with a 100 mm visual analogue scale (VAS) on satiety and fullness at baseline, midpoint and 12 weeks, which were based on those validated by Flint et al. (1).
2. Changes in self-reported dietary intake (total kilocalories, grams of carbohydrates and percent of total calories, grams of protein and percent of total calories and grams of fat

and percent of total calories, egg consumption) assessed via self-report (3-day dietary records) at baseline, midpoint and 12 weeks.
3. Changes in self-reported body weight and body mass index from baseline to 12 weeks.
4. Changes in blood markers (fasting insulin, high-sensitivity C-reactive protein, triglycerides, total cholesterol, HDL, LDL and VLDL) from baseline to 12 weeks.
5. Changes in carotenoid levels (lutein and zeaxanthin) from baseline to 12 weeks.
6. Changes in cognitive function from baseline to midpoint and 12 weeks.

Exploratory outcomes measures:

Exploratory outcomes will be analyzed similarly to the primary and secondary outcomes and include:

1. Self-reported physical activity scores measured at baseline, midpoint and 12 weeks assessed via GODIN Physical Activity Questionnaire.
2. Self-reported subjective norms, attitudes (both instrumental and affective attitudes – see below) perceived behavioral control, and intentions at baseline and 12 weeks via Theory of Planned Behavior Questionnaire. (4,5)

Instrumental attitudes:
- Useless–useful
- Unimportant–important
- Worthless–valuable
- Not worthwhile–worthwhile
- Harmful–beneficial

Affective attitudes:
- Unsatisfying–satisfying
- Unpleasant–pleasant
- Not enjoyable–enjoyable
- Boring–exciting
- Not fun-fun

SENSITIVITY ANALYSIS:

To explore robustness of the primary outcome, we will perform the following sensitivity analyses:

- We will perform sensitivity analyses on the primary outcome (change in HbA1c at 12 weeks) and secondary outcomes
    - including study site (UOW, UBC) as a covariate,
    - stratified by study site (UOW, UBC).
    - Per protocol analyses based on adherence to eating a compliant breakfast (i.e., uploaded daily breakfast photo that complies with group allocation, taking a conservative approach that if no photo is uploaded the breakfast did not comply) including only those participants who remained in the trial and had >80% adherence
    - Sub-analyses based on or controlling for breakfast egg intake

Secondary endpoints exploring within-group changes from baseline will be performed using a linear mixed model. The model will include fixed effects for timepoint (Baseline vs Week 6/12), stratified allocation factors (sex), site and random effects for participants to account for participant variability in the outcome measures.

HANDLING OF MISSING DATA AND OTHER DATA CONVENTIONS

No statistical imputations will replace missing data for the outcome measures (*data as observed*). However, the constrained baseline longitudinal data analysis – in a linear mixed model with restricted maximum likelihood – is a principled approach to addressing missing outcome data, including the baseline value of the continuous outcome measure. The model permits the inclusion of all participants in the analysis with either a baseline or a follow-up value. Thus, missing data will not be imputed for the ITT analyses. If a blood marker value is below the detection limit, we will explore the results following imputation of the value as limit of detection (LOD) divided by the square root of 2 (6).

REFERENCES

1. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Int J Obes Relat Metab Disord. 2000; 24:38–48.
2. Rabinovitz HR et al., Big breakfast rich in protein and fat improves glycemic control in type 2 diabetics. Obesity, 2014. 22(5): p. E46-E54.
3. Chang CR, Francois ME, and Little JP. Restricting carbohydrates at breakfast is sufficient to reduce 24-hour exposure to postprandial hyperglycemia and improve glycemic variability. The American journal of clinical nutrition, 2019. 109(5): p. 1302-1309.
4. Items developed based on Ajzen's recommendation for constructing scales to measure the Theory of Planned Behaviour (TPB) constructs taken from the appendix in Fishbein, M., & Ajzen, I. (2010). *Predicting and changing behavior: The reasoned action approach*. New York: Psychology Press.
5. Ajzen, I. (2010). Constructing a theory of planned behaviour questionnaire. Retrieved from: http://people.umass.edu/aizen/pdf/tpb.measurement.pdf (recently updated in 2019)
6. Canales RA, et al. Methods for handling left-censored data in quantitative microbial risk assessment. Appl Environ Microbiol 2018. 84(20): e01203-18.