The effect of acute exogenous oral ketone supplementation on blood glucose levels in type 2 diabetes

NCT 04194450
Version 7 – April 2, 2022 [H19-02947]

STATISTICAL ANALYSIS PLAN (SAP)

Exogenous Ketones in Type 2 Diabetes

Trial Registration: NCT04194450

Version 1: November 8, 2021 with dated adjustments up to April 2, 2022.

BACKGROUND AND AIM:

From December 2019 to November 2021, we completed the randomized crossover placebo-controlled study titled "Exogenous Ketones in Type 2 Diabetes". The aim of the trial was to determine whether acutely ingesting exogenous ketones, in the form of a ketone monoester drink (0.3 g/kg (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate, **Δ**G®, TDeltaS, Oxford, UK, provided by HVMN), can lower blood glucose in individuals with type 2 diabetes (T2D). The trial also aimed to examine the impact of exogenous ketones on brain/cognitive function, measures of inflammation, and appetite. The primary outcome was plasma glucose concentration after consumption of exogenous ketones compared to ingestion of placebo assessed over 180 minutes following supplement consumption. It was hypothesized that consumption of exogenous ketones would lower blood glucose compared to the placebo.

SAMPLE SIZE CONSIDERATIONS

At the time of study design, there were no known studies evaluating the effect of acute supplementation with exogenous ketones in individuals with T2D. Therefore, we estimated sample size based on our pilot study in healthy volunteers, in which we saw a ~15% reduction in glucose area under the curve after ketone supplementation compared to placebo [1]. Using means and standard deviations for fasting glucose (8.6 ± 2.3 mM) from our previously published study in people with T2D (N = 51) [2], a 15% reduction in blood glucose corresponds to an effect size of d = 0.73. Assuming 80% power with an alpha level of 0.05 and a moderate correlation between repeated measures of r = 0.7, a total sample size of N = 14 is needed to detect differences between ketone and placebo conditions (calculated using G*Power v3.1). To preserve power

and account for any dropouts or missing blood samples, we aimed to recruit 20 participants, aiming for equal males and females.

ANALYSIS SET AND STUDY POPULATION

The full analysis set of data will be derived from the set of all randomized participants in an intention-to-treat (ITT) analysis. 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. No statistical imputations will replace missing data for the outcome measures (*data as observed*), per contemporary guidelines [3]. However, the linear mixed effects model (see below for further details) is a principled approach to addressing missing outcome data. The model permits the inclusion of all participants in the analysis with a follow-up value (regardless of missing timepoints within a condition, or a missing alternative condition). Statistical analysis will be performed blinded to participant allocation.

OUTCOMES, STATISTICAL METHODS, AND COVARIATES

Primary endpoint: The preregistered primary outcome for this trial will be a comparison of blood glucose values assessed over 180 minutes following consumption of exogenous ketones or a placebo. Data will be analyzed according to the ITT principle via a linear mixed effects model with fixed effects for time after supplement ingestion (i.e., 30, 60, 90, 120, 150, 180 minutes), condition (i.e., ketone monoester vs placebo), period (depending on which condition was conducted first), and an interaction between time and condition and time and period as well as the baseline value (assessed at "time 0" immediately before consumption of the supplement) as a covariate. A random intercept for each participant (ID) and a random slope for time on the interaction of ID and period will be included. If a participant has a baseline value missing, we will impute this as the average (mean) baseline value observed across participants. Model assumptions of normality and linearity will be assessed visually by inspecting a Q-Q plot, and homoscedasticity will be assessed by plotting residuals vs fitted values. Models will be run using log-transformed outcome variables when departure from model assumptions is observed.

In addition to the primary analysis detailed above, we will explore the primary endpoint via the following confirmatory analyses:

1. Paired t-test on blood glucose incremental are under the curve (i.e., glucose above baseline fasting values across 180 minutes after ingestion of supplement or placebo)
2. Paired t-test on peak blood glucose concentration (i.e., highest blood glucose value measured after ingestion of supplement or placebo)
3. Paired t-test on peak blood glucose concentration above baseline (i.e., highest blood glucose value above fasting baseline after ingestion of supplement or placebo)
4. Paired t-test on blood glucose total area under the curve (i.e., glucose above zero across 180 minutes after ingestion of supplement or placebo)

Secondary outcome measures:

Secondary outcomes will be analyzed similarly to the primary outcome using a linear mixed effects model. Secondary and exploratory blood marker outcomes that are below or above the detection limit, will be imputed as limit of detection (LOD) / sqrt(2) or LOD * sqrt(2), respectively [4]. Preregistered secondary outcomes include:

1. Metabolic outcomes (plasma insulin, plasma c-peptide, plasma free fatty acids, plasma β-OHB)
2. Markers of brain/cognitive function (intracranial blood flow measured by ultrasound, blood pressure measured manually, cognitive function using BrainBaseline on an iPad, brain-derived neurotrophic factor)
3. Markers of inflammation (plasma tumour necrosis factor alpha, plasma interleukin-1β, plasma interleukin-6, total blood monocytes and monocyte subsets, monocyte histone acetylation)
4. Markers of appetite (self-reported hunger and fullness assessed via a 0 to 100 mm Visual Analog Scale, gastrointestinal symptoms assessed via a 0 to 100 mm Visual Analog Scale, total energy consumed in kilocalories during a buffet style meal at the end of testing)

Exploratory outcomes measures:

Exploratory outcomes will be analyzed similarly to the primary outcome using a linear mixed effects model. Exploratory outcomes that were not preregistered include:

1. Metabolomics
2. Electrolyte status, lactate, pH etc.

REPORTING RESULTS

We will summarize data (including participant baseline characteristics) as mean ± standard deviation (SD) for continuous and *N* (%) for categorical data. A significant effect will be concluded at two-sided *P*-value of < 0.05. We will report on the effect of condition and the interaction of the treatment effect over time. Effect estimates from the model alongside 95% confidence intervals around the mean differences in the outcome after accounting for the included covariates will be reported. Changes in outcome within condition (e.g., effect of ketone monoester on blood glucose at 180 minutes compared to baseline) will be reported via mean differences with SD outside the model, if applicable. Effect sizes (Hedges' g) will be stated.

PROGRAMMING PLAN

Statistical analysis will be performed after the last patient out, using R [5].

REFERENCES

1. Myette-Côté, É., et al., Prior ingestion of exogenous ketone monoester attenuates the glycaemic response to an oral glucose tolerance test in healthy young individuals. The Journal of Physiology, 2018. **596**(8): p. 1385-1395.
2. Francois, M.E., et al., Combined interval training and post-exercise nutrition in type 2 diabetes: a randomized control trial. Frontiers in Physiology, 2017. **8**: p. 528.
3. Chakraborty H. A mixed model approach for intent-to-treat analysis in longitudinal clinical trials with missing values [Internet]. 2009. Available at: http://www.rti.org/publication/mixed-model-

approach-intent-treat-analysis-longitudinal-clinical-trials-missing-values

4. Canales R.A., et al. Methods for handling left-censored data in quantitative microbial risk assessment. Appl Environ Microbiol 2018. **84**(20): e01203-18.
5. Team RC. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing 2020. Available at (accessed November 4, 2021): https://www.r-project.org/
6.