Acute Effects of Exogenous Ketone Ester Administration in Heart Failure

Statistical Analysis Plan

ClinicalTrials.gov Identifier: NCT04633460

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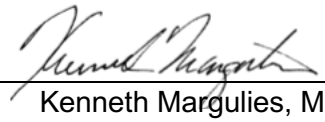
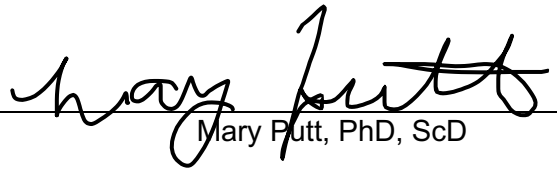


Kenneth Margulies, MD



Mary Putt, PhD, ScD

Study Design: Two-sequence, two-treatment individual-randomized crossover design.

Description of Baseline Characteristics: The demographic and clinical characteristics of the sample will be described overall (intention-to-treat) and by arm using descriptive statistics (means, medians, percentages) with measures of the variation (standard deviation (SD) and interquartile range (IQR)). Graphical procedures will be used to assess distributional assumptions.

Primary Analysis: The primary analysis will regress the difference in outcome for ketone versus placebo on treatment sequence. A Wald test of the intercept will be used for the hypothesis test of a treatment effect. The two co-primary outcomes are:

1. Peak VO_2 during incremental (maximal) exercise testing
2. Time to exhaustion during submaximal exercise testing at 75% peak workload

A type I error rate of 0.05 is pre-specified without correction for multiple comparisons.

Secondary Endpoints: The secondary outcomes will be analyzed using the same model. These are:

1. Percent change in systemic vascular resistance with maximal exercise testing at peak
2. Substrate utilization (reflected by the respiratory exchange ratio) during maximal exercise testing at peak
3. VO_2 efficiency (the ratio of total work performed over oxygen consumed above baseline during exercise, kJ/L of O_2) during submaximal cardiopulmonary exercise testing.

Exploratory Endpoints: Exploratory outcomes will be used to further understand possible mechanisms of action for the change in primary and secondary outcomes, and include several domains:

(a) Resting echocardiographic data: left ventricular ejection fraction, peak left ventricular global longitudinal strain, e' septal and lateral velocity, E/e' ratio, tricuspid annular planar systolic excursion, and the right ventricular s' velocity.

(b) Exercise hemodynamic data: Cardiac Output (heart rate x stroke volume), VE/VCO_2 slope, the arteriovenous O_2 content difference (quotient of VO_2 to cardiac output), mean arterial pressure, the vasodilatory reserve, and the mitral E to septal e' ratio assessed at:

- 25 watts of exercise during incremental study
- Peak of the incremental study
- 4 min during the submaximal study
- Time of exhaustion during the submaximal study

(c) Exercise metabolic data:

- Ventilatory Threshold (using the average of the V-slope method and the Ventilatory Equivalents method, when available)

- Respiratory Exchange Ratio assessed at:
  - 25W of exercise during incremental study
  - Peak of the incremental study
  - 4 min during the submaximal study
  - Time of exhaustion during the submaximal study

- Beta-hydroxybutyrate, glucose, lactate, non-esterified fatty acids, insulin, and glucagon assessed at:
  - 25W of exercise during incremental study
  - Peak of the incremental study
  - 4 min during the submaximal study
  - Time of exhaustion during the submaximal study

- Glucose kinetics during the submaximal study in the subgroup who received stable isotopes during the intervention visits:
  - Glucose rate of appearance
  - Glucose rate of disappearance

Safety Endpoint: Presence of exercise-induced arrhythmias (significant atrial or ventricular arrhythmias)

Physiologic Response to the Intervention: In addition to the primary and secondary outcomes, multiple other physiologic endpoints were collected, with some at multiple timepoints within each visit. For some endpoints, not all measurements occurred at regular intervals, and the number of measurements per subject varied. These analyses will be analyzed similarly to the primary endpoints, but with changes to address the repeated measurements within visits. In some cases, the responses can be summarized using a single summary statistic, e.g. the area under a curve (AUC) or the initial slope, while in other cases the individual measurements are of interest. Mixed effects models may be employed to efficiently answer questions related to outcome variables with repeated measurements.

Subgroup analyses:
- Biological sex
- Baseline peak VO_2 indexed to body weight (above versus below median value)
- Diabetic status (No versus Yes)
- Left ventricular ejection fraction above and below 60%[1]

A term for subgroup will be included in the model described above. A Wald-test of significance will be used to compare to the null value. This analysis is equivalent to the interaction analysis used in the analysis of parallel-group designs. Results will be presented graphically in a forest plot. Subgroup analyses are considered exploratory and thus no formal corrections for multiple comparisons will be used. Rather across the two primary outcomes, and with 4 subgroups, the expected number of tests with a p-value less than 0.05 is 0.4 hypothesis tests. More than one test with $p<0.05$ is suggestive of treatment effect heterogeneity.

Hypothesis generating analysis:
The achieved level of ketone in blood immediately prior to exercise is hypothesized to relate to each of the primary outcomes. This hypothesis will be explored graphically and using linear regression models with stratification by treatment sequence.

Power Calculations for Primary Endpoints:
Peak VO_2: With 18 subjects (9 per treatment sequence), the study has 91% power to detect a mean difference in peak VO_2 (mL/min/kg) between ketone and placebo of 1 mL/min/kg (effect size=0.83), assuming a standard deviation for the difference of 1.2 mL/min/kg.[2] The power calculation is based on a paired t-test with a two-sided type I error rate of 0.05.

Submaximal Exercise Endurance: Using the effect size noted in a study examining the impact of inorganic nitrate on submaximal exercise endurance in HFpEF,[3] a sample size of 18 participants has 88% power to detect an effect size of 0.78, using a paired t-test with a two-sided type I error rate of 0.05, assuming a correlation of 0.8 between measurements on the same individual.

A total of 21 participants were randomized anticipating loss to follow-up of around 3 participants (14%).

References

1. Rosch S, Kresoja KP, Besler C, Fengler K, Schober AR, von Roeder M, Lucke C, Gutberlet M, Klingel K, Thiele H, et al. Characteristics of heart failure with preserved ejection fraction across the range of left ventricular ejection fraction. *Circulation*. 2022;146:506-518
2. Zamani P, Rawat D, Shiva-Kumar P, Geraci S, Bhuva R, Konda P, Doulias PT, Ischiropoulos H, Townsend RR, Margulies KB, et al. Effect of inorganic nitrate on exercise capacity in heart failure with preserved ejection fraction. *Circulation*. 2015;131:371-380; discussion 380
3. Eggebeen J, Kim-Shapiro DB, Haykowsky M, Morgan TM, Basu S, Brubaker P, Rejeski J, Kitzman DW. One week of daily dosing with beetroot juice improves submaximal endurance and blood pressure in older patients with heart failure and preserved ejection fraction. *JACC Heart Fail*. 2016;4:428-437