Randomized, Controlled Dietary Treatment Study of Pediatric NAFLD

Statistical Analysis Plan

Funding Source: Nutrition Science Initiative

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Overview
Data will be collected at Emory University/Children's Healthcare of Atlanta and the University of California San Diego from July 2015 through July 2017. Primary and secondary outcome measures and a description of how these measures will be assessed are described in the protocol. No interim analysis is planned of the primary outcome. Randomization will be blocked by 4 subjects for intervention and control assignment and will be conducted by selecting sequentially numbered sealed envelopes. The study statistician will generate the random sequence, the investigators and coordinators will enroll the study subjects and will also open the envelope to reveal the assignment at the time of randomization. Study staff that will be blinded include the radiologists, scientists, and lab personnel that will be performing either the measurements for the MRI's and analyzing blood samples for the outcomes. A description of the methods for additional analyses can be found in the Statistical analysis plan.

General Statistical Considerations
Initial descriptive statistics will be calculated for the overall study cohort and each treatment arm separately. This will include histograms, means, medians, standard deviations and ranges for continuous variables to assess normality and identify potential outliers. Frequencies will be calculated for categorical variables. Treatment arms will be compared to each other on demographic and clinical characteristics collected at time of randomization. Two-sample t-tests will be used for normally distributed continuous variables, Wilcoxon rank-sum tests for non-normally distributed continuous variables, and Chi-square tests for categorical variables. In cases of small expected cell counts, exact tests may be used in place of Chi-square tests. For reporting inferential statistical, such as differences in means, 95 percent confidence interval will be used extensively to quantify the degree of clinical efficacy.

The extent of missing data will be assessed using the methods described above. All missing data will be verified by querying sites for missing information. We will examine the degree of randomness in missing data by comparing the frequency, reasons, pattern and time to dropout and missing values across treatment groups. Provided the data are missing at random (MAR), a likelihood based approach to analysis (such as linear mixed models) will be utilized to handle missing data.

Prior to modeling, outcomes will be assessed for normality using histograms and probability density plots. Data will be transformed prior to modeling to meet the assumption of normality. Residual plots by group will be inspected to assess heteroscedasticity. In cases where assumptions of normality are not met, log-transformed variables or square-root transformed variables will be used in analysis, or a non-parametric alternative will be employed.

Statistical analysis will be conducted using SAS v. 9.4 (Cary, NC) and all test will be two-sided. Statistical significance will be assessed at the 0.05 level unless otherwise noted.

Primary Outcome Measure
The primary outcome is percent change in hepatic fat content from baseline to 8 weeks in the intervention group compared to the control group. Percent change in hepatic fat will be compared between the two groups using two-sample t-tests or Wilcoxon-rank sum tests. The analysis will follow the intention to treat (ITT) principle, using all randomized participants in the analysis.

Secondary Outcome Measures
To estimate the intervention effect for all primary, secondary and post-hoc analyses, we will employ mixed models using baseline, week 4, and week 8 measurements conditioned on baseline values. This conditional joint response model is an extension to the traditional analysis of covariance model (ANCOVA), is more tolerant to missing data and is less biased than carrying forward baseline measurements. This modeling approach was chosen to adjust for possible differences between groups at baseline. Models will be constructed using the *PROC MIXED* procedure in SAS. Standard errors will be estimated using an unstructured covariance matrix and the Kenward-Roger method will be used to estimate the degrees of freedom for the fixed effects. Results from these models will be presented as differences in group means at week 8, adjusted for baseline, with associated 95% confidence intervals. All models will control for center as a fixed effect.

For the sweetness perception testing, mean model estimates of sweetness perception and pleasantness ratings at five different sucrose concentrations will be compared between study groups and visits using penalized B-splines due to the non-linear relationships between concentration and perceived sweetness and pleasantness. Patient specific random intercepts will be used to account for patient variation. Tukey's method will be used for adjustment for multiple comparisons. The concentration associated with the highest pleasantness rating will be determined using a repeated measures ANOVA with a group by visit interaction.