Cover Page for Statistical Analysis Plan

Official Title: The effects of added sugar intake on brain blood flow and hippocampal function in midlife adults

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Statistical Analyses

The aims for this study will be tested using General Linear Modeling. For hypotheses H1a and H2a, a Profile Analysis (multivariate within-in subjects ANOVA) will be performed. If the omnibus test is significant, univariate within subject ANOVAs will be performed, testing the effect of diet (baseline, low sugar, and high sugar diets) on each outcome. The multivariate extension is being used to control for type-I error inflation that might occur because each aim has three primary outcomes (Aim 1: 24-hour SBP, arterial compliance, and cerebrovascular reactivity; Aim 2: HC stiffness, HC damping ratio, and delayed recall memory). All assumptions for these models will be tested, and data will be screened for outliers and influential cases. Assumptions of linearity, sphericity, and normality will be evaluated using Box-Cox, Mauchly's, and Shapiro-Wilk tests, respectively. Because some values are expressed as ratios, if the assumptions of the within subject's ANOVA cannot be satisfied by transformations suggested by the Box-Cox test then either the non-parametric Friedman test or a Generalized Linear Model will be used. Given the cross-over design, the order effect will be tested using the same model but the effect will just be time point (baseline, post diet 1, and post diet 2). If significant, the order effect will be included in the final model. A significant diet effect will be followed by pairwise comparisons using Fisher's LSD procedure. The alpha is set at 0.05 for each test. Exploratory analyses will be conducted for hypotheses H1b and H2b, using regression models testing the changes (from baseline) in each primary outcome from Aims 1a and 2a after the high sugar diet with the changes in TGs, markers of oxidative stress, and inflammation. Additionally, regression models will be used to explore whether changes in the primary outcomes from Aim 1 moderate the relation between the changes in the primary outcomes in Aim 2. This moderation will be tested by including the interaction effects of changes in outcomes from Aim 1 and 2 with the changes in TGs, markers of oxidative stress, and inflammation. During Year 2, the PI will work with the COBRE Core's lead biostatistician (Dr. Pohlig) to conduct an interim data analysis for an R01 proposal. The O'Brien-Fleming alpha boundary function will be used, the alpha for significance of the interim analysis is .0054 and for the final analysis is .0492.

Randomization, Blinding, Power & Sample Size

To enhance scientific **rigor and reproducibilit**y, subjects will be randomized to diet order with Group A receiving the high sugar diet first followed by the low sugar diet and Group B receiving each diet in the opposite order. Investigators responsible for data collection or analysis will be blinded to treatment condition. To address **sex as a biological variable**, we will use a block randomization scheme to ensure an even number from each sex within each treatment group and will covary for sex in our analyses. A major objective is to generate preliminary data and conduct a power analysis for an eventual R01. Power for this pilot study was determined for the profile analysis for Aims 1 and 2, testing the change across the three measurement occasions for three outcomes. This study is adequately powered (power = .80), with n=30 to detect a moderate effect (d=.46), for three outcomes and three timepoints with nominal alpha (.05), assuming a conservative correlation among the outcomes and repeated measures of 0.3. If the multivariate test is significant, this study is adequately powered (power = .80), with n=30 to detect a moderate effect (d=.56), for three timepoints with nominal alpha (.05), assuming a correlation among the repeated measures of 0.3 for follow-up univariate within subject ANOVAs. To account for drop-out or experimental error, our total recruited sample size will be 40. This effect size matches ones seen in the literature, which found a moderately large effect (d = 0.69 with n=14) in the effect of increased serum TGs within 4-5 days of a high fructose meal[75].