STUDY PROTOCOL and STATISTICAL ANALYSIS PLAN

Human subjects Involvement, Characteristics and Design

The study will recruit adults from the Birmingham area for a low oxalate controlled diet. The individuals will be recruited, consented, and have blood samples collected at the UAB CCTS Clinical Research Unit. Sample processing, data acquisition and analysis will be performed in the Urology Stone Research Laboratory. A total of 60 participants is expected. An equal number of males and females is planned from all ethnic backgrounds.

- Inclusion criteria: mentally competent adults, who are able to read and comprehend the consent form (written in English); >18 and <70 yrs; BMI 20-25 (in the low BMI group); BMI 25-30 (in the overweight group); BMI >30 (in the obese group); good health as judged from a medical history without other medical comorbidities. individuals will need a normal blood comprehensive metabolic panel. Those with controlled hypertension (systolic blood pressure <160mmHg, diastolic <90 mmHg) and without severe dyslipidemia (LDL <200 mg/dL, HDL >30 mg/dL, and triglycerides <250 mg/dL) will be included.
- Exclusion criteria: history of any hepatic, renal (including kidney stone disease), bowel or endocrine disease (including diabetes) or any other condition that may influence the absorption, transport or urine excretion of ions, which will compromise the interpretation of results; abnormal blood metabolic profiles; poor 24 hour urine collections judged by 24 hour urine creatinine excretion (indicative of not collecting all urine in 24 hour period); pregnancy, intention to become pregnant in the near future, or lactation; <18 and > 70 years of age; BMI<20 ; GFR<60 ml/min/1.73 m2.
- Screening: fasted blood draw for comprehensive metabolic panel and lipid panel.
- Dietary Study: the subjects will consume for 4 days a controlled diet prepared in the metabolic kitchen of the CRU containing 16% protein, 30% fat and 54% carbohydrate, calcium (1000 mg), oxalate (50 mg) and other nutrients. Daily 24 hour urine collections will be performed on days 2-3-4. A fasting blood draw will be done on the morning of Day 5.

Statistical Analysis

Comparisons of means between groups, will be performed using the two-group t-test. In addition, the relationship between glycolate and oxalate will be examined using correlation analysis and linear regression analysis. Comparisons between groups will also be performed using analysis of covariance in order to account for any covariates of scientific interest. Distributions of continuous variables will be examined for normality using stem-and-leaf plots, normal probability plots, and the Kolmogorov-Smirnov test; variables that are determined to be non-normally distributed will either be log10 transformed prior to analysis, or will be analyzed using appropriate nonparametric methods such as the Wilcoxon rank-sum test or the Kruskal-Wallis test. All statistical tests will be two-sided, and differences will be considered statistically significant at $p < 0.05$.