Official Title: Oxalobacter Formigenes Colonization and Oxalate Excretion in Healthy Adults

NCT#: 03752684

Document Date: May 22, 2025

STUDY PROTOCOL

Objective of the study

The primary objective of the study was to determine if healthy adults can be colonized with Oxalobacter formigenes, a naturally occurring oxalate degrading intestinal bacterium, and if colonization results in a decrease in urinary oxalate excretion on a fixed diet.

Study Design

This study was designed as a sequential, diet-controlled study. The study recruited healthy adults from the Greater Birmingham area. The individuals were recruited, consented, and had blood samples collected at the UAB CCTS Clinical Research Unit. Sample processing, data acquisition and analysis was performed in the Urology Stone Research Laboratory.

- Inclusion criteria: not colonized with Oxalobacter formigenes, mentally competent adults without history of kidney stones, who are able to read and comprehend the consent form (written in English); >18 and <65 yrs; BMI 18.5-40 kg/m2; good health as judged from a medical history without other medical comorbidities. individuals will need a normal blood comprehensive metabolic panel.
- Exclusion criteria: colonized with Oxalobacter formigenes, 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 > 65 years of age; BMI<18.5 >40 kg/m2

Study Methods

Screening. The screening phase involved a fasted blood draw for comprehensive metabolic profile (CMP), two 24-hr urine collections (creatinine) and stool collections on self-choice diets. Screening blood tests were used as a health assessment, and urinary creatinine as measure of the candidates' ability to accurately collect urine. Stool at screening was used to test for *O. formigenes* colonization. If negative, participants were further tested after consuming a high oxalate containing meal.

Pre-Colonization Fixed Diet Study: Participants who passed screening collected stool and 24-hour urine specimens while ingesting a moderately high oxalate (210 – 240 mg/day) and low calcium (500 - 700 mg calcium/day) diet. For reference, a healthy diet rich in whole grain products, fruits and vegetables may contain close to 200 mg of oxalate per day, and the recommended dietary calcium intake per day for calcium oxalate kidney stone formers is 1000 mg. Twenty-four-hour urine and stool specimens were collected on days 3-5 of each dietary phase to allow for dietary equilibration. Lack of colonization with *O. formigenes* (pre-colonization phase) was again confirmed by stool culture.

Oxalobacter formigenes colonization procedure: Following completion of the pre-colonization controlled diet, participants ingested live *O.formigenes*,cells spread on a sandwich. One week later participants collected stool for colonization culture testing.

Post-Colonization Fixed Diet Study: Successfully colonized participants repeated stool and urine collections on the same controlled diet.

Assays: Urine and stool oxalate was measured in urines and stool by ion chromatography coupled with mass spectrometry.

Statistical Analysis Plan

The primary outcome measure was decrease in urinary oxalate content after colonization with O. formigenes on the fixed diet. The secondary outcome was decrease in stool oxalate content after colonization with O. formigenes on the fixed diet.

The means of oxalate content of repeated urine and stool collections for each phase (pre and post colonization) were used, and the decrease in oxalate content after colonization determined for each participant. Paired comparisons were performed using linear mixed-effects models (for repeated measures). The covariates included gender, body mass index, age, and a dichotomous variable denoting loss of colonization during the follow-up period (adjusted analyses); these covariates were included because of potential associations with endogenous oxalate synthesis or gut permeability and absorption. Statistical tests were two-sided and were performed using a significance level of 5%. Statistical analyses were performed using SAS software (version 9.4; SAS Institute, Cary, NC). PROC MIXED of SAS was used to perform linear mixed effects models analyses. The sample size was selected based on feasibility considerations for this pilot Basic Experimental Study in Humans (BESH).