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Title of study: Assessment of Endogenous Oxalate Synthesis

IRB-151020005

NCT05229952

Document date: 12/08/24

STUDY PROTOCOL

Objective of the study

The objectives of this study were to determine endogenous oxalate synthesis rates by continuous intravenous infusion of $^{13}C_2$-oxalate (a stable isotope of oxalate) and to identify surrogate methods of estimating this parameter using urine collections on a low-oxalate normal calcium fixed diet.

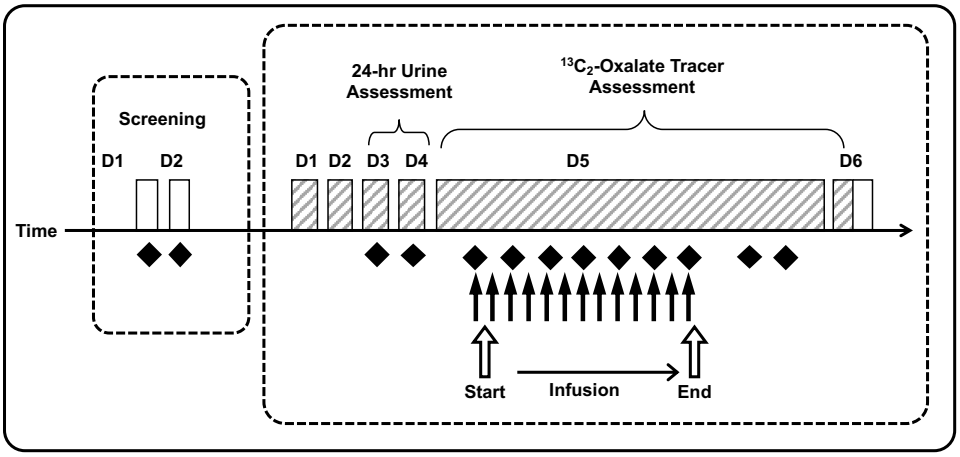
Study Design

The study recruited adults from the Birmingham area for a low oxalate controlled diet. 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. A total of 20 participants was expected. An equal number of males and females was planned from all ethnic backgrounds. The study corresponded to a BESH trial (Basic Experimental Study in Humans). All enrolled participants received the same intervention defined as a period of fixed diet followed by the infusion of $^{13}C_2$-oxalate.

- Inclusion criteria: mentally competent adults, who are able to read and comprehend the consent form (written in English); >18 and <75 yrs; BMI 18.5-50 kg/m2; good health as judged from a medical history without other medical comorbidities. individuals will need a normal blood comprehensive metabolic panel. With of without history of calcium oxalate kidney stones
- 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 > 75 years of age; BMI<18.5

Study Methods

- The study sequence is profiled in figure below.



Study sequence overview. *After a screening period on self-choice diet, which included two 24-hr urine collections (♦) and a blood draw, participants ingested a fixed low-oxalate, normal calcium diet for a total of 5 days (shaded boxes). The $^{13}C_2$- oxalate infusion was performed in the fasted state, following 4 days of dietary equilibration and two 24-hr urine collections. Participants collected one pre-infusion hourly urine, and 6 hourly urines during the infusion, and a post infusion 6-hr and 12-hr collection, the aggregate comprising a 24-hr collection. Blood (↑) was collected before the infusion and every 30 minutes during the infusion. Consumption of the fixed low oxalate diet was resumed at the end of the 6-hr infusion and the study ended at the completion of the 24-hr urine collection on the morning of day 6.*

- Screening. A fasted blood was drawn for comprehensive metabolic panel. Two 24-hour urine were collected on self-choice diets to assess participant's skill at completing 24-hourb urine collections. Body composition was measured by impedance (segmental body composition estimated by impedance, BC-

418 segmental body composition analyzer, TANITA). A DEXA scan was performed at the end of the study to assess body composition more accurately.

- Fixed Diet and infusion Study:
- Participants ingested fixed, isocaloric diets (2000 or 2500 kcal/day) containing a low amount of oxalate (30 - 50 mg/day), normal calcium (1000 - 1120 mg/day), and normal ascorbic acid intake (90-125 mg/day) for 5 consecutive days. The low-oxalate diet was prepared in a metabolic kitchen at UAB. Participants were asked to refrain from vigorous exercise during the study. After 2 days of dietary equilibration, two 24-hr urine were collected, followed by a primed, steady-state infusion of $^{13}C_2$-oxalate.
- On Day 5, following a 10-hr overnight fast, and waking at 6:00 a.m., participants emptied their bladders and drank 500 ml of water. Participants were admitted to the Clinical Research Unit (CRU) at 7:00 a.m to start the oxalate infusion.
- Primed, $^{13}C_2$-oxalate, continuous, intravenous infusion. The intravenous infusions were initiated at 8:00 a.m. with a priming dose of sodium $^{13}C_2$-oxalate administered over a 10 min period in 0.9% saline (equivalent to a 1.5 to 2 hr dose). This was immediately followed by a constant infusion of $^{13}C_2$-oxalate for up to 6 hrs (0.022 µmol/kg/h) in the fasted state. Separate catheters on opposite arms were used for the infusion of $^{13}C_2$-oxalate and blood collections. Serial urine (hourly) and plasma (every 30 min) samples were be collected between 1 hour prior to and 6 hours after the start of the infusion. The remainder of the 24-hr urine that day was collected until the following morning. Urine output was maintained during the infusion by having participants drink 200 ml of bottled water per hour. Blood and urine samples were collected every half-hour and hour, respectively. At the end of the infusion (6 hrs), food consumption was resumed and the remainder of the 24-hr urine for that day was collected. Final concentrations of $^{13}C_2$- oxalate in 0.9% saline infusion bags were measured by ion chromatography coupled with mass spectrometry, and the recovery of intravenously infused $^{13}C_2$-oxalate in urine collections was calculated both during the steady-state period and the 24 hours following initiating infusion.
- Assays. ^{12}C- and ^{13}C- isotopomers of oxalate in urine, plasma and food extracts were measured by Ion Chromatography coupled with Mass Spectrometry. Urine and plasma sample handling and storage follow strict protocols to avoid crystallization of oxalate or vitamin C breakdown to oxalate (leading to under or overestimation of oxalate). The analysis of 24-hr urine collections included: volume, urine oxalate measurement by Ion Chromatography coupled with Mass Spectrometry, and creatinine.

STATISTICAL ANALYSIS PLAN

The primary outcome measure was endogenous oxalate synthesis as measured by the $^{13}C_2$-oxalate continuous infusion, measured in mg/day.

The formula for the calculation endogenous oxalate synthesis oxalate is

Oxalate Synthesis Rate (mg/day) = {i x [(Ei / EOx) – 1]} x 90.02 x 24 hours/1000

where "i" is the tracer infusion rate in µmol/hr; (Ei /EOx) is the ratio of the $^{13}C_2$-oxalate mole percent isotopic enrichment of the $^{13}C_2$-oxalate infusion solution (Ei: 99.6%) and the mole percent enrichment of plasma oxalate (EOx); and 90.02 g/mol represents the molecular mass of oxalate.

The secondary outcome measures were the mean 24-hr urinary oxalate excretions on the low-oxalate fixed diet measured in mg/day and in mg oxalate/g creatinine.

Exploratory endpoints were the association between endogenous oxalate synthesis rate, 24-hr urinary oxalate excretion on the fixed diet, body composition and hourly fasting urinary oxalate excretion.

Equilibration of plasma $^{13}C_2$-oxalate over time was analyzed by repeated-measures one-way ANOVA. Comparison of endogenous oxalate synthesis between groups (BMI <30 vs ≥30 kg/m^2, male vs female) was done with t-test. The linear relationships of endogenous oxalate synthesis with urinary creatinine and lean mass was assessed separately by ordinary least squares regression. Pearson correlation coefficients were calculated between endogenous oxalate synthesis, urinary creatinine, lean mass and gender. SAS software Version 9.4 was used in these analyses. The criterion for statistical significance was $p<0.05$ and all tests two-sided.

The sample sizes were selected based on feasibility considerations for this pilot Basic Experimental Study in Humans (BESH).