SUPPLEMENTAL MATERIALS

Endogenous oxalate synthesis and urinary oxalate excretion

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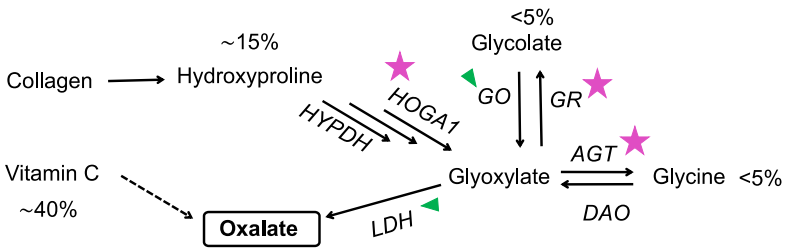
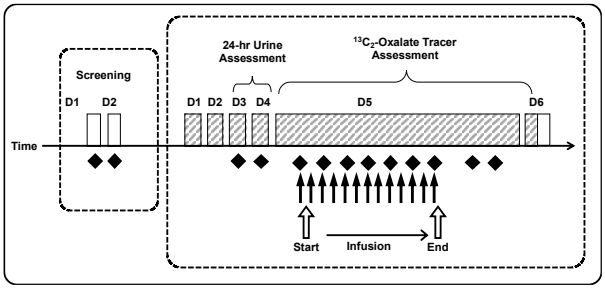
Supplemental Figure S1. Pathways of endogenous oxalate synthesis. *AGT: alanine:glyoxylate aminotransferase, LDH: lactate dehydrogenase, GO: glycolate oxidase, GR: glyoxylate reductase, HOGA1: 4-hydroxy-2-oxoglutarate aldolase, HYPDH: hydroxyproline dehydrogenase. DAO: D-amino oxidase. Vitamin C to oxalate is non enzymatic. AGT and GO are liver-specific, while GR and HOGA1 are highly expressed in liver and kidney. %: contribution of precursors in healthy non-stone forming human controls. Stars denote the enzymes that are deficient in primary hyperoxaluria (PH): AGT in PH1, GR in PH2, HOGA1 in PH3. Green arrows denote therapeutic targets for substrate reduction in oxalate synthesis.*



Supplemental Methods

Study Participants

Twenty adult participants (12F/8M) were enrolled in the study. Exclusion criteria included BMI <19 kg/m^2 or BMI >45 kg/m^2, eGFR <60 ml/min/1.73m^2, history of nephrolithiasis, primary hyperoxaluria, bariatric surgery, inflammatory bowel disease, other causes of enteric hyperoxaluria, primary hyperparathyroidism, uncontrolled hypertension or dyslipidemia, diabetes requiring insulin, pregnancy or lactation, known liver or cardiac disease, and use of supplements including ascorbic acid. Screening studies included a serum comprehensive metabolic profile (CMP), and assessment of accuracy of 24-hr urine collections based on creatinine excretion. A subset of participants (n=12) received a dual energy X-ray absorptiometry (Lunar iDXA, GE Healthcare) scan to assess body composition. All had segmental body composition estimated by impedance (BC-418 segmental body composition analyzer, TANITA). Demographics (gender, race and ethnicity) were self-reported. The study sequence is profiled in figure 2. Participants ingested fixed, eucaloric diets containing a low amount of oxalate (30 - 40 mg/day), normal calcium (1000 - 1100 mg/day), and normal ascorbic acid intake (90-125 mg/day) (Sup. Table S1, S2) for 4 consecutive days. Two 24hr urine specimens were collected after 2 days of equilibration on the fixed diet. Dietary compliance was evaluated by measuring urinary chemistries (creatinine, oxalate, sulfate, chloride, phosphorus, ammonium, potassium, magnesium, citrate) on the two 24-hr collections on the fixed diet. Participants were asked to refrain from vigorous exercise during the study. The study was approved by the University of Alabama at Birmingham Institutional Review Board. Written informed consent was obtained from all participants in the study. All procedures were perfomed in accordance with the 1964 Helsinki Declaration, and with ethical standards of the institution. The study qualified as Basic Experimental Studies in Humans (BESH) and was registered on ClinicalTrials.gov as NCT05229952.



Supplemental Figure S2. Study protocol 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.*

Primed, $^{13}C_2$-oxalate, continuous, intravenous infusion

The study sequence is profiled in figure 2. Participants ingested fixed, eucaloric diets containing a low amount of oxalate (30 - 40 mg/day), normal calcium (1000 - 1100 mg/day), and normal ascorbic acid intake (90-125 mg/day) (Sup. Table S1, S2) for 4 consecutive days. The $^{13}C_2$- oxalate infusion was performed in the fasted state, following 4 days of dietary equilibration and two 24-hr urine collections. 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 and collected one pre-infusion hourly urine. 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). Separate catheters on opposite arms were used for the infusion of $^{13}C_2$-oxalate and blood collections. Urine output was maintained during the infusion by having participants drink 250 ml of bottled water per hour. Blood and urine samples were collected every half-hour and hour, respectively, for a total of 6 post-infusion urines and 12 post-infusion blood samples. 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.

Supplemental. Table S1. *Low oxalate menu composition. Nutrient composition was calculated with the Nutrition Data System for Research (NDSR) software. In addition, the oxalate content of the meals was assessed in the research lab, by acid extraction followed by measurement with the isotope dilution technique and ion chromatography coupled with mass spectrometry. Calcium and vitamin C content of the food was kept within recommended daily intakes. Animal protein intake, although high, was within reported range in the population.*

2000 kcal menu	**Total**		**Breakfast**		**Lunch**		**Dinner**	
Energy (kcal)	1997		709		646		642	
Fat (g)	65	[29%]	24	[30%]	12	[26%]	22	[30%]
Carbohydrates (g)	257	[50%]	107	[58%]	89	[54%]	61	[38%]
Protein (g)	102	[21%]	19	[12%]	33	[20%]	49	[32%]
Animal protein (g)	74							
Sodium (mg)	2479		595		1184		699	
Vitamine C (mg)	111		48		58		6	
Calcium (mg)	1021		592		178		250	
Oxalate (mg, NDSR)	39		11		23		5	
Oxalate (mg, lab)	36							

2500 kcal menu	**Total**		**Breakfast**		**Lunch**		**Dinner**	
Energy (kcal)	2496		879		701		916	
Fat (g)	82	[29%]	34	[34%]	20	[24%]	28	[28%]
Carbohydrates (g)	317	[50%]	122	[53%]	99	[56%]	96	[42%]
Protein (g)	130	[21%]	26	[13%]	37	[20%]	67	[30%]
Animal protein (g)	95							
Sodium (mg)	2513		695		1235		584	
Vitamine C (mg)	124		49		65		10	
Calcium (mg)	1096		628		195		272	
Oxalate (mg, NDSR)	43		12		23		8	
Oxalate (mg, lab)	32							

Supplemental. Table S2. *Low oxalate menus plans. Menus were attributed to individuals based on their caloric requirements.*

MENU: 2000 Kcal		**MENU: 2500 Kcal**	
Breakfast	**Amount**	**Breakfast**	**Amount**
Egg, boiled	1 large	Egg, boiled	2 large
Waffles, plain	2 medium	Waffles, plain	2 medium
Pancake syrup	1 container (40 g)	Pancake syrup	1 container (40 g)
Butter, regular	5 g	Butter, regular	5 g
Strawberries, frozen	1/2 cup (110 g)	Strawberries, frozen	1/2 cup (110 g)
Apple juice	8 FO	Apple juice	12 FO
Whole milk	8 FO	Whole milk	8 FO
Lunch		**Lunch**	
White bread	2 slices	White bread	2 slices
Deli Turkey	90 g	Deli Turkey	90 g
Mustard	1 pkg	Mustard	1 pkg
Mayonnaise, real	1 pkg	Mayonnaise, real	1 pkg
Broccoli, frozen	1/2 cup (80 g before cooking)	Broccoli, frozen	1/2 cup (80 g before cooking)
Green eyed peas, frozen	1 cup (240 g before cooking)	Green eyed peas, frozen	1.5 cup (320 g before cooking)
Promise margarine	10 g	Promise margarine	10 g
String cheese, regular	1 stick	String cheese, regular	1 stick
Apple	1 medium	Apple	1 medium
Dinner		**Dinner**	
Chicken breast	120 g, after cooking	Chicken breast	170 g, after cooking
Butter	5 g	Butter	5 g
Instant rice (in microwaveable cup)	1 white cup (46 g, before cooking)	Instant rice (in microwaveable cup)	1.5 white cup (46 g, before cooking)
Iceburg lettuce	1/8 head wedge	Iceburg lettuce	1/8 head wedge
Kraft Ranch dressing	1 pkg (7/16 oz)	Kraft Ranch dressing	1 pkg (7/16 oz)
Canned peaches, juice packed	4 oz	Canned peaches, juice packed	8 oz
Salt, regular	1 pkg	Salt, regular	1 pkg
Pepper	1 pkg	Pepper	1 pkg
Bottled water	3 (16.9 oz) bottles	Bottled water	3 (16.9 oz) bottles

Metabolic Analysis of Oxalate Using Isotope Infusion and Calculations

The measurement of Endogenous Oxalate Synthesis (EOS) used the common technique of constant infusion of the tracer with a priming dose to shorten the time to isotopic steady-state. At isotopic equilibrium, the various pools containing the oxalate tracer can be simplified to a single pool into which tracer enters and from which sampling occurs (Sup. Fig. S3).

The rate of appearance of oxalate (Ra) can be calculated with Steele's steady-state equation

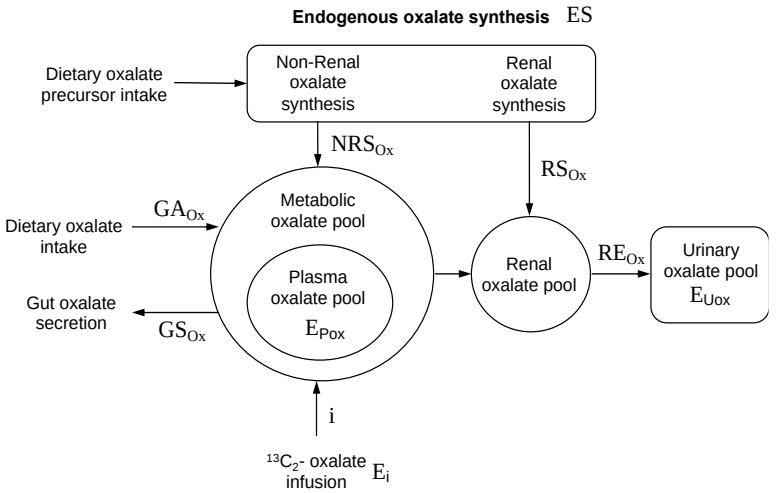
$$Ra = Rd = Q = i\,[(E_i / E_{Ox}) - 1] \qquad \text{Equation [1]}$$

with

$$\text{Rate of Appearance } (GA_{Ox} + ES_{Ox}) = \text{Rate of Disappearance } (GS_{Ox} + RE_{Ox}) \qquad \text{Equation [2]}$$

where

Ra is the rate of appearance
Rd is the rate of disappearance,
Q is the flux of oxalate (μmol/kg/h)
i = is the $^{13}C_2$-oxalate infusion rate (μmol/kg/h)
E_i = isotopic enrichment of the $^{13}C_2$-oxalate infusion (99.6%)
E_{Ox} = mole percent enrichment of the oxalate plasma pool with $^{13}C_2$-oxalate at isotopic plateau.
GA_{Ox} = is the gut absorption rate of oxalate
ES_{Ox} = is the Endogenous Synthesis rate of Oxalate and can be conceptualized into the sum of non-renal synthesis and renal synthesis of oxalate (ES_{Ox} = NRS_{Ox} + RS_{Ox})
GS_{Ox} = is the gut secretion rate of oxalate
RE_{Ox} = is the renal excretion rate of oxalate



Supplemental Figure S3. Schematic representation of the oxalate isotope model of analysis.

Oxalate is an end-product of metabolism. In the fasted state and with normal renal function, the rate of appearance (Ra) Ra reflects synthesis. Furthermore, in the absence of significant renal oxalate synthesis, which would dilute the $^{13}C_2$-tracer with new $^{12}C_2$-oxalate, the enrichment of oxalate in urine is equal to that in plasma. Thus, urinary oxalate mole percent enrichment (MPE) can be used for the calculation of endogenous oxalate synthesis oxalate (EOS, in mg/24 hr) using the equation:

$$EOS\ (\mu mol/kg/h) = i\,[(E_i / E_{Uox}) - 1]$$

or

$$EOS\ (mg/day) = i\,[(E_i / E_{UOx}) - 1] \times 90.02 \times 24 \times \text{Body weight (kg)} / 1000 \qquad \text{Equation [3]}$$

using (E_{uOx}), the excess mole percent enrichment of the total urinary oxalate pool with $^{13}C_2$-oxalate (referred to as "enrichment" or "MPE" in the text and independent of complete urine collections.), 90.02 the molecular mass of oxalate (g/mol), 24 referring to 24 hours, BW: body weight in kg.

For the estimated endogenous oxalate synthesis rate based on fasted hourly urine oxalate excretion, in the absence of net gut oxalate absorption (GS_{Ox} + GA_{Ox} = 0) and under the same conditions as above, the renal excretion of oxalate in the fasted state reflects endogenous oxalate synthesis and Equation [2] can be simplified to

$$EOS = RE_{Ox}$$

Thus

$$\text{Estimated EOS (mg/d)} = 24 \times U_{Ox\ fast}\ (mg/h) \qquad \text{Equation [4]}$$

With $U_{Ox\ fast}$ (mg/h) the mean hourly urinary $^{12}C_2$-oxalate excretion during the infusion, independent of the infusion.

It is worth noting that the use of urine MPE $^{13}C_2$-oxalate instead of plasma MPE to determine endogenous oxalate synthesis offers several advantages, including, 1), the high concentration of oxalate in urine compared with plasma increases the accuracy of MPE measurements, and 2) reliance on plasma oxalate requires sub-micromolar limits of quantification, currently challenging with IC/MS based techniques, and rigorous sample handling to avoid breakdown of ascorbic acid (present at 10-100 fold greater concentrations compared to plasma oxalate) to oxalate, which is facilitated by pH>5, UV light, temperature and hemolysis, and leads to overestimation of plasma $^{12}C_2$-oxalate concentrations, underestimation of plasma $^{13}C_2$-oxalate MPE and ultimately overestimation of oxalate synthesis rates. Data supporting the assumptions mentioned is presented in Sup. Table S8 and Sup. Fig S4-S6. Detailed data is presented in Sup. Tables S8-S10 and Sup. Fig S4-S14.

Statistical analysis

Pharmacokinetic steady-state equilibration was analyzed over time (from 4 to 6 hours) by repeated-measures one-way ANOVA. Comparisons of endogenous oxalate synthesis (primary outcome variable), oxalate Ra, urinary oxalate, and estimated endogenous oxalate synthesis (all secondary outcome variables) between groups (BMI <30 vs ≥30 kg/m^2, male vs female) were done using the two-group t-test. We determined that there were no statistically significant differences between the two BMI groups for all four of these outcome variables. The linear relationships of endogenous oxalate synthesis and the secondary outcome variables with other continuous variables, including urinary creatinine and lean mass, were assessed by simple and multiple variable linear regression analyses. Covariates such as gender or body weight were included in some of the regression models. Correlation analyses were performed using Pearson correlation analysis, with correlation analyses involving gender performed using Spearman correlation analysis. For example, correlation coefficients were calculated between endogenous oxalate synthesis, urinary creatinine, lean mass, and sex, and were tested for statistical significance. Outcome variables (all were continuous) were determined to be normally distributed through using box plots, stem-and-leaf plots, normal probability plots, and the Kolmogorov-Smirnov test. Sensitivity analyses were performed for the variables based on DXA measurements with multiple imputation under the assumption that that missing DXA values were missing at random. The criterion for statistical significance was p<0.05 and all tests were two-sided. Data are expressed as mean ± SD or median [range], unless otherwise indicated. Statistical analyses and generation of graphs and plots were performed using SAS software, version 9.4 (SAS Institute, Cary, NC), and GraphPad Prism version 9 (GraphPad Software, Boston, MA).

Chemicals

All reagents were of the highest analytical grade and obtained from either Sigma-Aldrich Chemicals (St Louis, MO) or Fisher Scientific (Pittsburgh, PA). The sodium $^{13}C_2$-oxalate for infusion was obtained from Cambridge Isotopes (Andover, MA), and compounded for intravenous infusion in an FDA-approved 503B facility (Pine Pharmaceuticals, NY).

Sample preparation

Urine samples were diluted two-fold in 100 mM HCl (final pH <2) before freezing at -80°C to prevent calcium oxalate crystallization and potential de-novo oxalogenesis from ascorbic acid breakdown. Blood was collected in sodium heparin tubes, immediately placed on ice, protected from light, and centrifuged within 1 hr at 2000 g, 4°C, for 10 min. Plasma samples were stored at -80°C within 2 hours of blood collection. Plasma that showed evidence of hemolysis was discarded, as hemolysis may lead to the breakdown of ascorbic acid to oxalate. For $^{12}C_2$-oxalate analysis, plasma was diluted four-fold in sodium $^{13}C_2$-oxalate internal standard (final concentration of 4 μM $^{13}C_2$-oxalate) and filtered using Amicon Ultra 10 kD nominal molecular mass limit filters (Millipore Sigma, Burlington, MA). For determination of excess mole percent enrichment of $^{13}C_2$-oxalate in plasma, samples were diluted two-fold in water, prior to centrifugal filtration. For $^{12}C_2$-glycolate analysis, plasma was diluted four-fold in sodium $^{13}C_2$-glycolate internal standard (final concentration of 5 μM $^{13}C_2$-glycolate) prior to centrifugal filtration. Centrifugal filters were cleaned with 0.01 M HCl to eliminate trace levels of organic acids, including oxalate and glycolate. Plasma samples were centrifuged at 15,000 g, 4°C, for 30 min and the ultra-filtrate immediately acidified to a final concentration of 30mM with 2M HCl. Acidified ultrafiltrates were stored in a cooled autosampler (10°C) and analyzed within 24-hrs. For ascorbic acid determination, plasma samples were extracted with 5% meta-phosphoric acid/1mM EDTA, as previously described by Bayram et al..

Analytical methods

Oxalate and glycolate isotopomers were measured by ion chromatography coupled to mass spectrometry (IC/MS). The IC/MS equipment consisted of an ISQ-EC single quadrupole mass spectrometer coupled to a Dionex ICS-5000 ion chromatograph with refrigerated AS-AP auto-sampler (10 μl injection loop) and gradient pump (all Thermo Fisher Scientific Inc., Waltham MA). The equipment was controlled with Chromeleon 7.2.10 software. The oxalate assay utilized an IonPac 5 μm AS22, 2x250 mm, anion exchange column fitted with AG22 guard (2 x 50 mm), operated at 30°C, flow rate 0.3 ml/min, and ammonium carbonate as the mobile phase (20-33 mM linear gradient over 30 minutes). Mass detector parameters are found in Sup Table S3. The column eluent was mixed with 50% acetonitrile in water at 0.1 ml/min using a zero-volume tee before entry into the mass spectrometer. Selected ion monitoring (SIM) was used to determine oxalate isotopomers using the following mass/charge ratios: SIM89 ($^{12}C_2$-oxalate) and SIM91 ($^{13}C_2$-oxalate). For quantification of excess mole percent enrichment of $^{13}C_2$-oxalate, samples were diluted in water and analyzed against standards with known amounts of $^{13}C_2$-oxalate and $^{12}C_2$-oxalate in the range of 0% to 50% $^{13}C_2$-oxalate excess mole percent enrichment. The

limits of detection (LOD) of the urinary oxalate method were 1 picomole for $^{12}C_2$-oxalate and $^{13}C_2$-oxalate, and 0.2% for $^{13}C_2$-oxalate excess mole percent enrichment. Plasma glycolate was measured by IC/MS using $^{13}C_2$-glycolate isotope dilution, as previously described (Fargue et al. 2018). The IC/MS glycolate assay used an AS15, 2 x 250 mm, anion exchange column with potassium hydroxide as column eluent at a flow rate of 0.3 ml/min, which was mixed with 50% acetonitrile at a flow rate of 0.3 ml/min prior to entry into the ISQ-EC mass spectrometer. (Dionex Integrion HPIC system and ISQ EC mass spectrometer, Thermo Fisher Scientific, Waltham, MA) (Fargue et al. 2018, Knight et al. 2011). Urinary creatinine was measured using an EasyRA chemical analyzer (Medica, Carolina Chemistries Corp., NC). Plasma ascorbic acid was measured by High Performance Liquid Chromatography with coulometric detection as described by Bayram and colleagues, using a Kinetex C18, 2.6 µm, 100x4.6 mm column (Phenomenex, Aschaffenburg, Germany) with 50mM sodium phosphate, pH 3.0 as mobile phase at a flow rate of 0.4 ml/min.

Supplemental Table S3. Mass detector parameters for oxalate and glycolate analysis

Analysis	Oxalate	Glycolate
Ion chromatography		
Column	IonPac AS22 IC Column, 2x250 mm	IonPac AS15 IC Column 2x250 mm
Guard	IonPac AG22 Guard Column, 2x50 mm	IonPac AG15 Guard Columns 2x50 mm
Column mobile phase	0.3 ml/mn NH4CO3 [20-33mM]	0.3 ml/mn KOH [3-80mM]
Suppressor	None	ADRS 600 Anion Suppressor (2 mm)
Before entry into MS, addition	0.1 ml/mn 50% Acetonitrile/water	0.3 ml/mn 50% Acetonitrile/water
Mass spectrometer	ISQ-EC	ISQ-EC
Mass detector	Negative ESI mode	Negative ESI mode
Sheath gas pressure	42.9 psig	56.0 psig
Auxilliary gas pressure	4.8 psig	6.5 psig
Sweep gas pressure	0.1 psig	0.1 psig
Vaporizer temperature	227°C	338°C
Ion transfer tube temperature	250°C	250°C
Source voltage	-700V	-1500V
Source CID voltage	10V	15V
mass/charge ratio recorded	SIM89, SIM91	SIM75, SIM77

The IC/MS equipment consisted of an ISQ-EC single quadrupole mass spectrometer coupled to a/ for oxalate analysis: a Dionex ICS-5000 ion chromatograph; b/ for glycolate analysis: Dionex Integrion HPIC system; both with refrigerated AS-AP auto-sampler (10 µl injection loop) and gradient pump; and the equipment controlled with Chromeleon 7.2.10 software (all Thermo Fisher Scientific Inc., Waltham MA).

Plasma oxalate assay by IC/MS

The plasma oxalate assay used $^{13}C_2$-oxalate isotope dilution and ion chromatography coupled with mass spectrometry (Sup. Table 4). A partial validation of the method was performed and detailed below. Plasma oxalate levels were found to be low in all cases (< 0.7 µM) and below the lower limit of quantification of the assay in the majority of cases (Sup. Table S10).

1. Sample processing

Ascorbic acid breakdown to oxalate in serum or plasma is known to be promoted by higher temperature, UV rays and high pH, leading to overestimation of oxalate in samples handled incorrectly. In addition to keeping plasma protected from light and at 4-10°C, other sources of oxalate contamination during processing can be traced to the type of tubes used for blood collection and can be as high as 70 nmol for ACD solution A tubes. Heparin tubes (sodium or lithium heparin) were found to contain the least amount of oxalate contamination (<0.2 nmol) and performed better than potassium EDTA tubes (0.7 nmol) or serum tubes (0.2-1 nmol). Sodium heparin tubes were therefore used for the study.

Supplemental Table S4. Overview of the plasma oxalate assay by IC/MS

Analyte		**Oxalate**
Matrix		**Human plasma**
Sample storage temperature		-70°C
Sample preparation		
	Blood collection	Sodium heparin tube
	Sample handling	On ice, protected from light
	Plasma sample volume required	80 uL
	Diluent solution	$^{13}C_2$-oxalate (4uM) in ultrapure deionized water
	Sample dilution	Final dilution 1:4
	Sample clean-up	Filtration (diluted plasma + internal standard)
	Fillters used	Amicon Ultra 10 K MWCO, precleaned with 0.01 M HCl
		Acidification of ultrafiltrate (30mM HCl final)
Post-preparation stability		4-10°C, up to 24 hours
Detection method		Ion Chromatography Mass Spectrometry
	Column	IonPac 5 um AS22, 2x150, with AG22 guard
	Elution buffer	Ammonium carbonate, 20-33 mM
		Acetonitrile/Water 50/50% added at entry into MS
	Volume injected	10 uL
Analysis		
	Regression	Linear regression
	LOB (plasma method)	0.19 uM
	LOD (plasma method)	0.23 uM
	LLOQ (injected, above blank)	0.12 uM (1.2 pmol)
	LLOQ for plasma oxalate assay	0.5 uM

LOB: limit of blank, LOD: limit of detection, LLOQ: lower limit of quantification.

2. Precision and accuracy

Precision and accuracy were determined on standards (in water) and plasma samples. The min-to-max error for plasma samples above LLOQ was plus/minus 0.25 µM plasma oxalate concentration.

3. Sensitivity

The lower limit of quantification (LLOQ) was determined as 0.12 µM injected above blank (1.2 pmol) using the cut-off of CV < 20%, translating as a plasma oxalate level of 0.5 µM. The upper limit of quantification (ULOQ) was not determined for this assay.

Supplemental Table S5. Precision and accuracy of the plasma oxalate assay by IC/MS

Precision and Accuracy	Within-Run		Between-Days	
	%CV	% accuracy	%CV	% accuracy
0.25 uM standard			16%	8%
0.5 uM standard			7%	1%
0.2 uM standard	11%	9%	29%	-7%
0.4 uM standard	6%	5%	7%	-3%
1 uM standard	5%	3%	4%	1%
Plasma Qc (0.9 uM nominal, 0.225 uM injected)	14%	10%	12%	-2%

CV: coefficient of variation

A comparison of sample processing using ultrafiltration vs extraction of oxalate in plasma by protein precipitation with trichloroacetic acid (TCA) was done. Briefly, TCA, (10% final concentration) was added to plasma mixed with the internal standard $^{13}C_2$-oxalate, excess TCA removed by mixing with Freon/Trioctylamine before dilution (final 1:4) and analysis by IC/MS. The two methods yielded comparable results, showing that there is no significant oxalate binding to protein and loss of oxalate using the filtration method; however, sensitivity and precision were much lower with the TCA extraction method because of a higher blank (Sup. Table 5).

Supplemental Table S6. Comparison of ultrafiltration and TCA extraction method.

	UF method	TCA method
LOB	0.2 uM	0.7 uM
Plasma Qc, measured	1.2 uM	1.9 uM
Plasma Qc, within-run CV (n=6)	10% CV	58% CV
Paired comparison, n=8 plasma lots	p = 0.53	(paired t-test)

LOB: limit of blank, UF: ultrafiltration, TCA: trichloroacetic acid, CV: coefficient of variation

Supplemental Table S7. Recovery in the plasma oxalate assay by IC/MS

Recovery	%difference
Sample Plasma (0.25 uM injected spike)	10-50%
Sample Plasma (0.5 uM injected spike)	<10%
Sample Plasma (1 uM injected spike)	<10%

4. Recovery

Recovery of oxalate was assessed by spiking plasma samples with known amounts of $^{12}C_2$-oxalate and compared to oxalate standards prepared in water at the same spike levels (Sup. Table S7). Recovery was greater than 90% at spiked oxalate levels above 0.5µM.

5. Stability

Post-preparative stability was assessed in a cooled autosampler tray (4-10°C) and samples were stable over the course of 24 hours. Freezer storage stability (-70°C) was assessed up to 5 years on a single plasma sample. There was a 18% difference between Year 5 and the initial value (+0.16 uM). Another sample was assessed over 2 years with 7% difference (-0.06 uM) with the initial value.

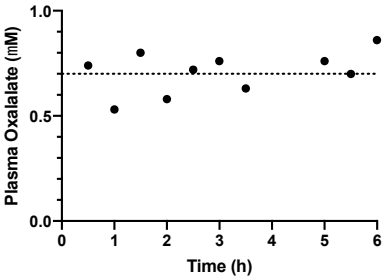
Supplemental Data

Supplemental. Table S8. *Repeated infusions in two healthy volunteers.*

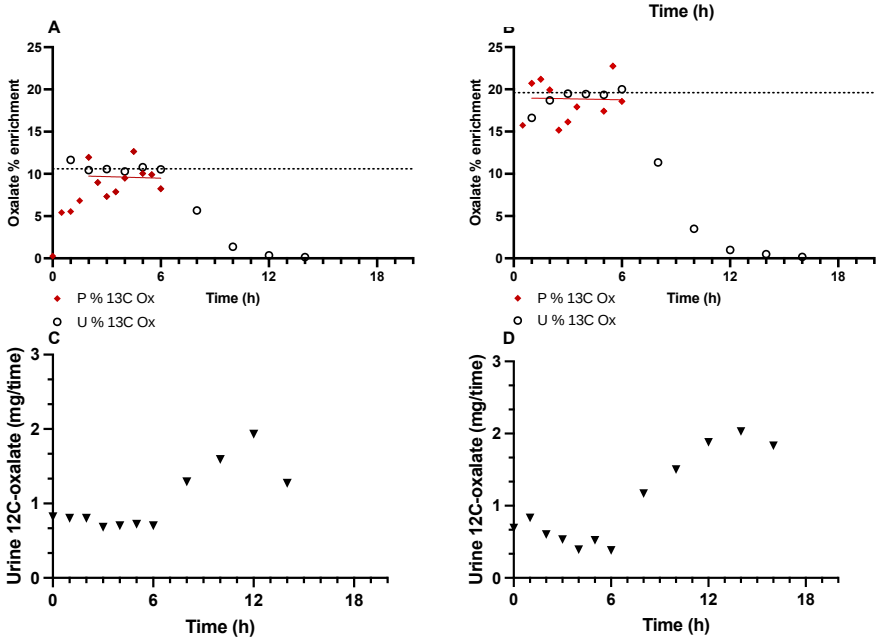
Individual	A		B	
Age (years)	45		25	
Gender	female		male	
Oxalate infusion				
Infusion rate (nmol/hr/kg)	12	27	29	29
24-hr 13C2-oxalate recovery (%)	113%	83%	74%	104%
Mean hourly 13C2-oxalate recovery (%)	111%	71%	67%	105%
Mean urinary 13C2-oxalate MPE (%)	11	21	19	20
Mean plasma 13C2-oxalate MPE (%)	10	18	ND	ND
Endogenous oxalate synthesis (mg/day)	15.2	14.8	23.5	22.5
Endogenous oxalate synthesis (mg/day/kg lean mass)	0.31	0.30	0.33	0.32

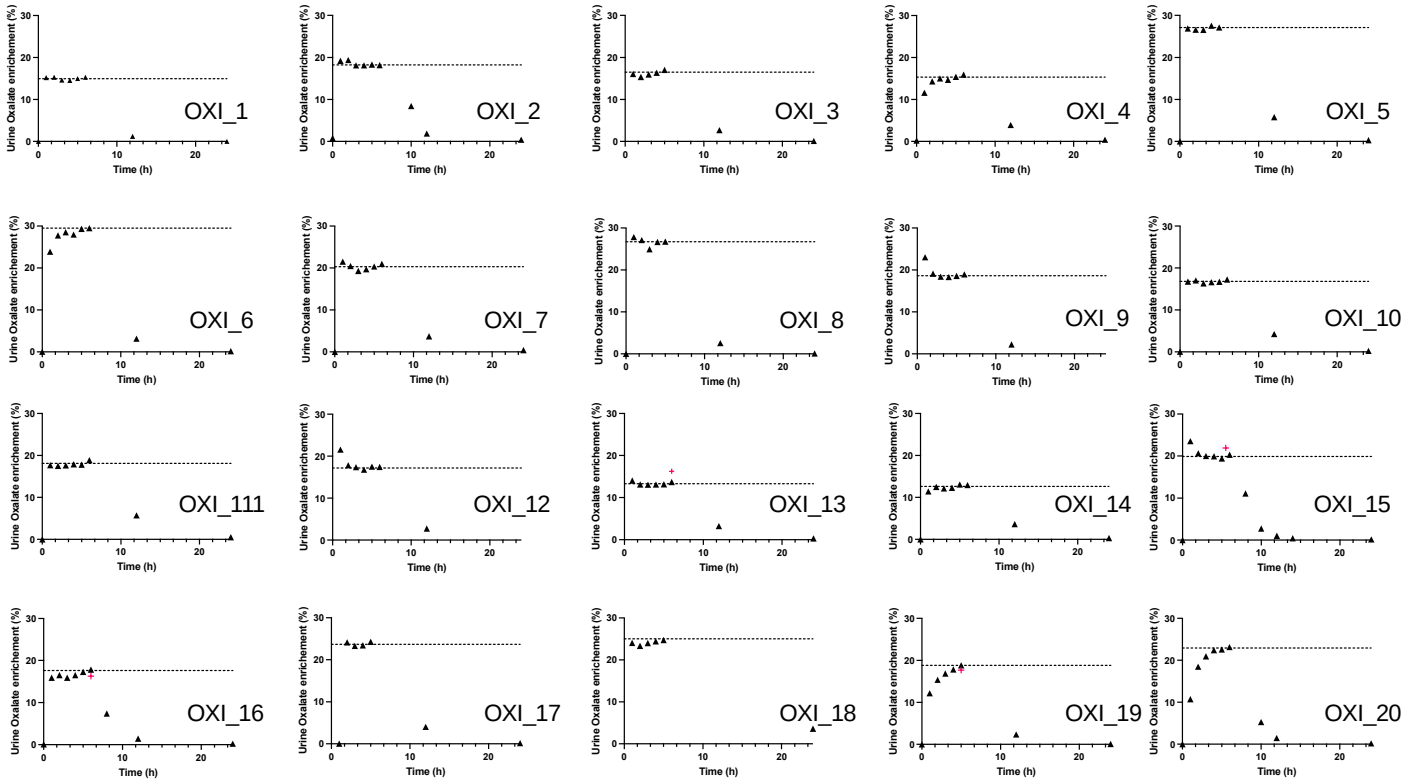
The primed, constant infusion of $^{13}C_2$-oxalate was done twice in two participants within a 2-month period to evaluate the reliability of the procedure. The calculated oxalate synthesis using the isotope tracer method was reproduceable in the repeats and both low and high infusion doses gave similar results.

Supplemental Figure S4. Repeated plasma oxalate measures over the course of the infusion of oxalate. *Blood samples were collected every 30 minutes over the course of the 6-hr infusion, in the fasted state, and $^{12}C_2$-oxalate was measured by IC/MS (see methods). Data is shown for participant A at the high infusion rate (Sup. Table 6). There was no significant change in oxalate over the infusion (0.7 ± 0.1 μM, CV 14%).*



Supplemental Figure S5. Full pharmacokinetic of the primed, constant infusion of $^{13}C_2$-oxalate at two doses in the one healthy volunteer. *Plasma (red diamonds) and urine (white circles) mole percent enrichments of oxalate (A, B) and timed urinary oxalate excretion (C, D, hourly during the infusion, every 2 hours for 8 hours and overnight collection to complete a full 24-collection since the start of the protocol) are shown over time in participant A (Sup. Table 6) at two doses (low infusion rate: A, C; high infusion rate: B, D). Steady-state was achieved within 3 hours for both urine and plasma (linear regression line shown for plasma measures in red, as stated in Methods, the variability of the assay is greater in plasma than in urine (coefficient of variability for plasma enrichments =13-18% for plasma vs < 2% in urine). There was no difference in mean oxalate enrichments between plasma and urine at steady-state (p = 0.11 and 0.55 with Welch's t-test for low and high infusion rates, respectively).*





Supplemental Figure S6. Pharmacokinetic of urine $^{13}C_2$-oxalate mole percent enrichment following the primed, constant infusion of $^{13}C_2$-oxalate. *Steady-state was achieved in all participants by the end of the infusion, allowing for repeat measurements except for participant OXI_19. Steady-state urine$^{13}C_2$-oxalate mole percent enrichment is indicated by the dotted line. Mean plasma enrichment at steady-state is indicated by the red cross for the five study participants in which it was measured (total plasma oxalate ≥ 0.5 μM and lack of hemolysis on samples at steady-state). There was no significant difference in plasma and urine enrichments at steady-state (paired t-test = 0.77).*

Supplemental Table S9. Pharmacokinetic parameters derived from the $^{13}C_2$-oxalate infusion.

Healthy Volunteers	Method	N	Miscible pool		Biological half-life	N	Plasma Ox	FE ox	Clearance of oxalate	Oxalate synthesis rate	
			(mg)	(umol)	(hr)		(uM)		(ml/min)	(mg/d)	(umol/d)
Elder [JCI, 1960]	14C-oxalate	3	7.2 4.9-8.6	80 39-71	2.5 [2.2 - 2.8]		[0.5 - 1.2]			34 - 63	378-700
Hodgkinson [Clin Sci, 1974]	14C-oxalate	3	4.8 ± 1.7	53 ± 19	1.5		1.4 [1.3-1.6]	1.95 [1.4 - 2.6]	249 [162-358]		
Hautmann Invest. Urol 1979	14C-oxalate	6	3.7 ± 0.5 [1.8 - 5.3]	41 ± 6 [20- 59]	1.5 ± 0.1 [1.2 - 2.3]		1.2 [0.5 - 1.6]	2.4 ± 0.5 [1.6 - 2.7]	252 [182 - 344]	37 ± 4 [22 - 58]	411 ± 44 [244 - 644]
Prenen Proc EDTA, 1979	14C-oxalate	3			2.7 [2.6 - 3]		[0.1 - 0.9]	2.3 [1.8 - 2.6]	175 -279		
Current Study	13C2-oxalate	4 [6]	2.3 ± 0.8 [1.4 - 3.2]	26 ± 8 [16 - 36]	1.9 ± 0.3 [1.7 - 2.2]	20 [8]	0.6 ± 0.1 [<0.5 - 0.7]	1.9 ± 0.4 [1.47 - 2.58]	255 ± 59 [173 - 342]	17 ± 4 [11 - 24]	189 ± 44 [122-267]

The half-life of oxalate was determined by single phase decay analysis of urine $^{13}C_2$-oxalate MPE after the end of the infusion in 4 participants (6 sets of data including repeated infusions) who collected more than 3 urines (3-6) post-infusions. The miscible pool of oxalate (Q_{OX}, µmoles) was calculated as

$$Q_{OX} = (F \times 100) / (Ep_{OX} \times k)$$

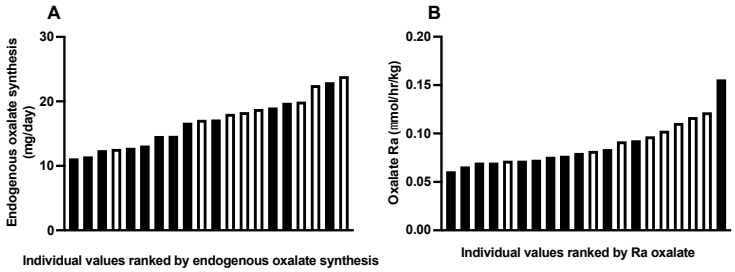
The Clearance of Oxalate (Cl_{OX}, ml/min) was calculated in all 20 participants as

$$Cl_{OX} = (F / C_{OXss})*1000 / 60$$

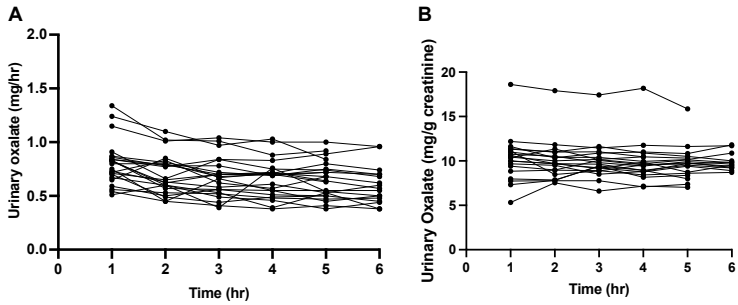
Where F is the $^{13}C_2$-oxalate infusion rate (µmole/hr), k is the elimination constant (hr^{-1}, estimated from the post-infusion urine collections), Ep_{OX} is $^{13}C_2$-oxalate MPE in urine at steady-state (%), C_{OXss} is the estimated concentration of $^{13}C_2$-oxalate in plasma at steady-state (µM), which was calculated as

$$C_{OXss} = (P_{OX} \times Ep_{OX}) / (100 - Ep_{OX})$$

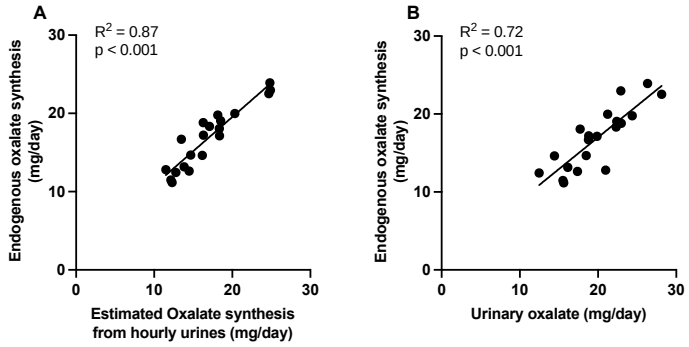
with P_{OX} (µM): mean plasma $^{12}C_2$-oxalate during the infusion. The molecular mass of $^{12}C_2$-oxalate is 90.02 g/mol. The miscible pool of oxalate is the amount of body oxalate the $^{13}C_2$-oxalate tracer mixed into.



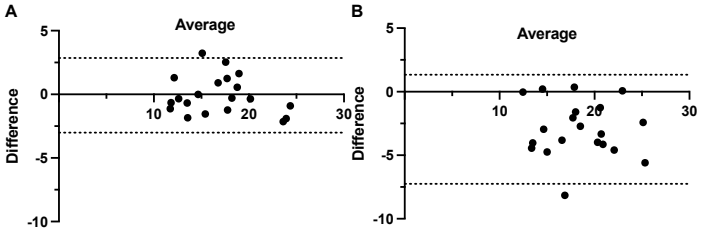
Supplemental Figure S7. Individual endogenous oxalate synthesis rates. *Oxalate synthesis rate was calculated with the $^{13}C_2$-oxalate isotope tracer infusion method (Equation 1 in Methods). White bars: males, black bars: females.*



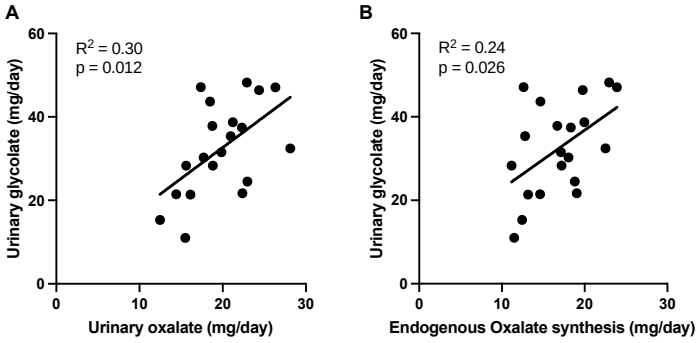
Supplemental Figure S8. Urinary oxalate excretion over the course of the $^{13}C_2$-oxalate isotope infusion*. Urinary excretion of $^{12}C_2$-oxalate was stable over time: (A) mg oxalate/hr and (B): mg oxalate/g creatinine (p=0.43 and 0.55 for A and B, respectively, 1-way ANOVA).*



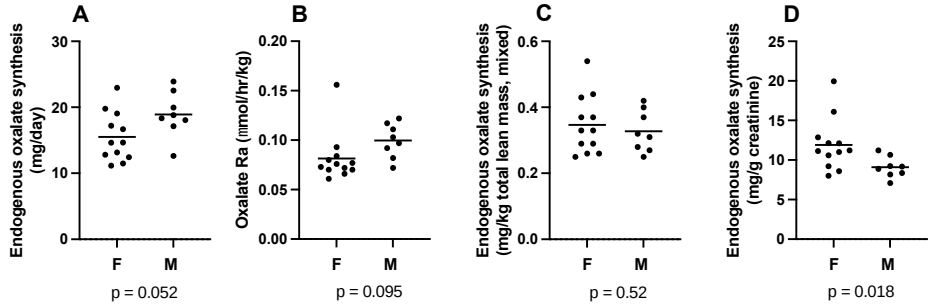
Supplemental Figure S9. Relationship between oxalate synthesis and urinary oxalate measurements. *Endogenous oxalate synthesis rate calculated with the $^{13}C_2$-oxalate isotope tracer infusion method was plotted against estimated 24-hr oxalate synthesis using the fasted hourly urinary oxalate excretion rate (A) or the mean of two 24-hr urinary oxalate excretions on a fixed low oxalate diet (B).*



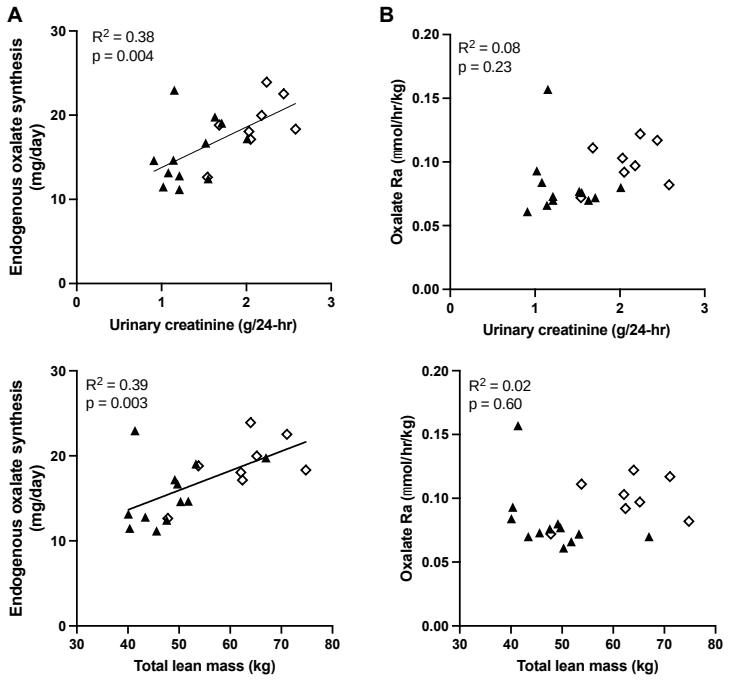
Supplemental Figure S10. Bland-Altman plots comparing endogenous oxalate synthesis by the isotope tracer method and the fasted hourly urine collections (A) and 24-hr urine collection on the controlled diet (B). *Limits of agreements are indicated by the dotted lines, bias for the method using fasted urine collection is -0.1, and -2.9 for the 24-hr urine collections on the diet.*



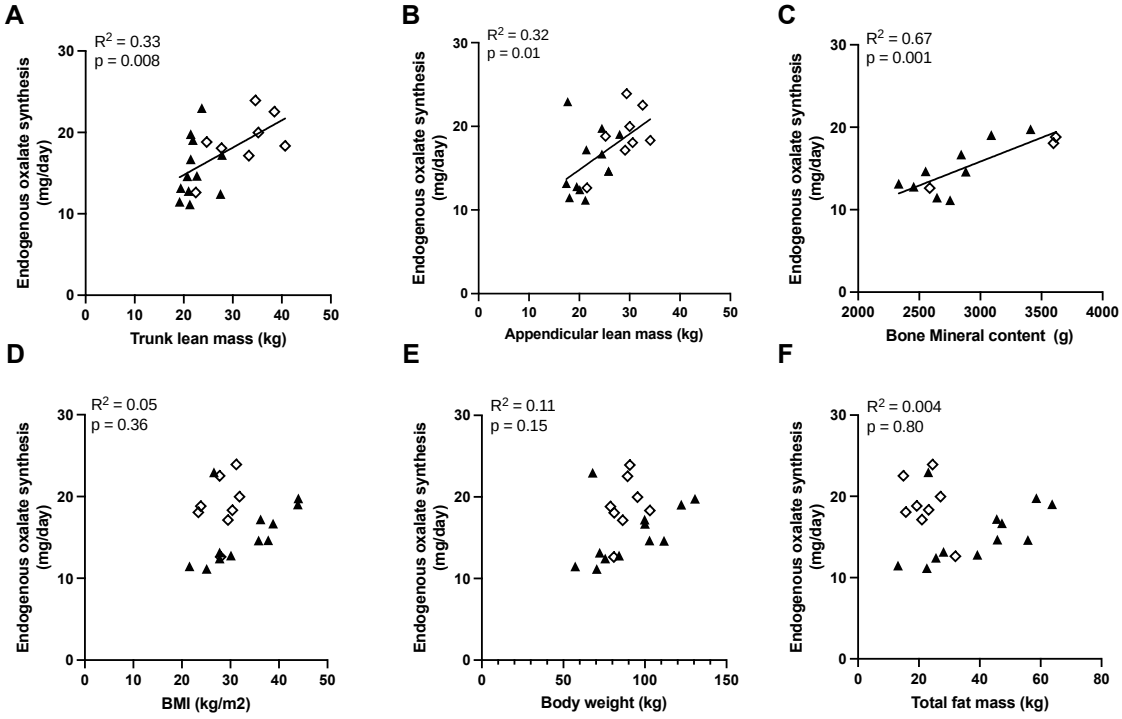
Supplemental Figure S11. 24-hr urinary glycolate and oxalate under controlled diet. *Mean 24-hr urinary glycolate excretion on the controlled diet was plotted against mean 24-hr urinary oxalate excretion on the controlled diet (A) and against endogenous oxalate synthesis determined by the isotope infusion (B).*



Supplemental Figure S12. Endogenous oxalate synthesis in female and male participants. *Median endogenous oxalate synthesis rate (A) and rate of appearance of oxalate (B) calculated with the $^{13}C_2$-oxalate isotope tracer infusion method, endogenous oxalate synthesis adjusted for total body lean mass (C) or 24-hr urinary creatinine (D) for male (M) and female (F) participants. The difference in endogenous oxalate synthesis between female (N=12, mean ± SD = 15.5 ± 3.7 mg/day) and male (N=8, mean ± SD = 18.9 ± 3.4 mg/day) was not significant, (p=0.052). After adjusting for lean mass, the difference decreased between sexes (mean ± SD = 0.323 ± 0.081 vs 0.303 ± 0.043 mg/kg lean mass in female and males, respectively, p = 0.52)*



Supplemental Figure S13. Endogenous oxalate synthesis, lean mass and urinary creatinine excretion. *Endogenous oxalate synthesis rate and rate of appearance (Ra) were calculated with the $^{13}C_2$-oxalate isotope tracer infusion method and plotted against mean 24-hr urinary creatinine excretion on the low oxalate fixed(A, B) or against total body lean mass as estimated by segmental body composition with DXA scan or bio-impedance (C, D), In addition to a significant positive correlation between endogenous oxalate synthesis and lean mass and urinary creatinine excretion, there was also a significant association between gender and urinary creatinine (Table S10). Black triangles: females, White diamonds: males.*



Supplemental Figure S14. Endogenous oxalate synthesis and anthropometric parameters. *Endogenous oxalate synthesis rate calculated with the $^{13}C_2$-oxalate isotope tracer infusion method was plotted against trunk lean mass (A), appendicular lean mass (B), bone mineral content (C), BMI (D), body weight (E) and total fat mass (F). Body composition parameters were estimated by segmental body composition with DXA scan or bio-impedance (A, B, F) pr DXA scan (C). There was significant association between gender and lean mass (R^2=0.47, p<0.001). Black triangles: females, White diamonds: males.*

Supplemental Table S10. Results of regression analyses for selected study variables and outcome measures.

Regression analyses	Variable	EOS		estimated EOS		24-hr urinary oxalate		Rate of appearance of oxalate	
		R2	*p*	R2	*p*	R2	*p*	R2	*p*
Independent (predictor) variable	gender	R^2 = 0.194	*0.052*	R^2 = 0.222	*0.036*	R^2 = 0.196	*0.051*	R^2 = 0.147	*0.095*
	urinary creatinine	R^2 = 0.382	*0.004*	R^2 = 0.302	*0.012*	R^2 = 0.350	*0.006*	R^2 = 0.077	*0.235*
	lean mass	R^2 = 0.387	*0.003*	R^2 = 0.308	*0.011*	R^2 = 0.381	*0.004*	R^2 = 0.016	*0.597*
	appendicular lean mass	R^2 = 0.316	*0.010*	R^2 = 0.260	*0.022*	R^2 = 0.267	*0.020*	R^2 = 0.007	*0.732*
	trunk lean mass	R^2 = 0.330	*0.008*	R^2 = 0.350	*0.006*	R^2 = 0.263	*0.021*	R^2 = 0.137	*0.108*
	fat mass	R^2 = 0.004	*0.802*	R^2 = 0.037	*0.415*	R^2 = 0.002	*0.866*	R^2 = 0.402	*0.003*
	bone mineral content	R^2 = 0.672	*0.001*	R^2 = 0.586	*0.004*	R^2 = 0.274	*0.081*	R^2 = 0.276	*0.080*

Regression analyses	Variable	covariate	EOS			estimated EOS			24-hr urinary oxalate		
			R2	*p*	*model p*	R2	*p*	*model p*	R2	*p*	*model p*
Independent (predictor) variables	urinary creatinine	gender	R^2 = 0.383	*Ucreat = 0.036* *gender = 0.853*	*p = 0.016*	R^2 = 0.310	*Ucreat = 0.160* *gender = 0.667*	*p = 0.043*	R^2 = 0.350	*Ucreat = 0.061* *gender = 0.990*	*p = 0.026*
	lean mass	gender	R^2 = 0.388	*l.mass = 0.033* *gender = 0.912*	*p = 0.015*	R^2 = 0.324	*l.mass = 0.129* *gender = 0.534*	*p = 0.036*	R^2 = 0.382	*l.mass = 0.037* *gender = 0.881*	*p = 0.017*
	appendicular lean mass	gender	R^2 = 0.321	*a.l.mass = 0.092* *gender = 0.729*	*p = 0.037*	R^2 = 0.287	*a.l.mass = 0.231* *gender = 0.436*	*p = 0.057*	R^2 = 0.281	*a.l.mass = 0.175* *gender = 0.577*	*p = 0.061*
	trunk lean mass	gender	R^2 = 0.330	*t.l.mass = 0.081* *gender = 0.904*	*p = 0.033*	R^2 = 0.352	*t.l.mass = 0.082* *gender = 0.797*	*p = 0.025*	R^2 = 0.272	*t.l.mass = 0.201* *gender = 0.652*	*p = 0.067*
	lean mass	body weight	R^2 = 0.388	*l.mass = 0.013* *b.w = 0.889*	*p = 0.015*	R^2 = 0.321	*l.mass = 0.016* *b.w. = 0.574*	*p = 0.037*	R^2 = 0.382	*l.mass = 0.014* *b.w. = 0.896*	*p = 0.017*

Urinary creatinine, lean mass, appendicular lean mass, trunk lean mass and bone mineral content were significantly associated with EOS, estimated EOS and 24-hr urinary oxalate on the low-oxalate diet. Gender was not strongly associated with these measures on its own, but may influence these relationships.

Supplemental Table S11. Demographics and infusion data for participants by gender.

	Females			Males		
Demographics	*mean ± SD*	*median*	*range*	*mean ± SD*	*median*	*range*
N		12			8	
Gender (M/F)		F			M	
Race/ethnicity AA/C (H)		10 / 2			1 / 7 (1)	
Age (yrs)	43 ± 11	44	[24-63]	35 ± 10	34	[24-54]
Body mass index (BMI< kg/m2)	33 ± 7	33	[22-44]	28 ± 3	29	[23-32]
Body weight (kg)	91 ± 24	92	[57-131]	88 ± 8	88	[79-103]
Total body fat mass (kg)	39 ± 17	42	[13-64]	22 ± 6	22	[15-32]
Total body lean mass (kg)	48 ± 7	48	[40-67]	63 ± 9	63	[48-75]
Trunk lean mass (kg)	22 ± 3	21	[19-28]	32 ± 7	34	[23-41]
Appendicular mass (kg)	22 ± 4	21	[17-28]	29 ± 4	30	[21-34]
Baseline, Low-oxalate diet						
Plasma oxalate (µmol/L)			[<0.5 - 0.56]			[<0.5 - 0.67]
Plasma glycolate (µmol/L)	5.1 ± 0.9	4.9	[3.5-6.8]	4.3 ± 0.6	4.1	[3.8-5.7]
Plasma ascorbic acid (µmol/L)	56 ± 18	57	[27-81]	56 ± 10	56	[38-69]
Renal fraction of excretion of oxalate [a]	1.8 ± 0.6	1.4	[1.4-2.5]	2 ± 0.2	1.8	[1.7-2.3]
Renal fraction of excretion of glycolate	0.5 ± 0.2	0.60	[0.3-0.8]	0.4 ± 0.1	0.40	[0.3-0.7]
Urinary oxalate (mg/day)	**18 ± 4**	**19**	**[12-24]**	**22 ± 4**	**22**	**[17-28]**
Urinary oxalate (mg/g creatinine)	14 ± 3	15	[8-20]	11 ± 2	11	[9-14]
Urinary glycolate (mg/day)	30 ± 12	28	[11-48]	36 ± 8	35	[25-47]
Urinary glycolate (mg/g creatinine)	23 ± 10	24	[10-42]	18 ± 6	16	[13-30]
Urinary creatinine (g/day)	1.3 ± 0.3	1.2	[0.9-2.0]	2.1 ± 0.4	2.1	[1.5-2.6]
Oxalate Infusion day						
Mean hourly 13C2-oxalate recovery (%)	94 ± 11	97	[72-109]	96 ± 8	99	[83-106]
Mean urinary 13C2-oxalate MPE (%)	20 ± 5	18	[15-29]	19 ± 5	19	[13-25]
Mean plasma 13C2-oxalate MPE (%) [a]						
Urinary oxalate (mg/hr), T60-T360	0.64 ± 0.16	0.59	[0.48-1.0]	0.80 ± 0.16	0.77	[0.60-1.03]
Urinary oxalate (mg/g creatinine) T60-T360	10 ± 3	10	[8-18]	9 ± 2	10	[7-11]
Ra oxalate (umol/hr/kg)	0.082 ± 0.025	0.075	[0.061-0.156]	0.995 ± 0.02	1.0	[0.072-0.122]
Endogenous oxalate synthesis (mg/day)	**16 ± 4**	**15**	**[11-23]**	**19 ± 3**	**19**	**[13-24]**
Estimated 24-hr oxalate synthesis (mg/day), with hourly fasting urines	**15 ± 4**	**14**	**[11-25]**	**19 ± 4**	**18**	**[14-25]**
Endogenous oxalate synthesis (mg/day/kg lean mass)	0.323 ± 0.08	0.295	[0.245-0.555]	0.303 ± 0.043	0.299	[0.245-0.374]
Endogenous oxalate synthesis (mg/day/g creatinine)	12 ± 3	11	[8-20]	9 ± 1	9	[7-11]

The two groups are defined by gender, M: male, F: female, AA: African American/Black, C: Caucasian/White, H: Hispanic or Latino, Urinary oxalate and glycolate excretions were calculated as the mean of two 24-hr urine collections under controlled diet. Mean hourly oxalate recovery and urinary MPE were calculated during the last 3-4 steady-state hourly periods and estimated 24-hr oxalate synthesis using mean hourly oxalate excretion at steady state. For adjusted endogenous oxalate synthesis rates, the mean of the two 24-hr urinary creatinine excretion on the controlled diet was used, body composition was measured by DXA-scan or bio-impedance.

Supplemental Table S13. Demographics and infusion data for participants by BMI group

	BMI<30 kg/m2			BMI>30 kg/m2		
Demographics	*mean ± SD*	*median*	*range*	*mean ± SD*	*median*	*range*
N		10			10	
Gender (M/F)		5 / 5			3 / 7	
Race/ethnicity AA/C (H)		6 / 4 (1)			7 / 3 (0)	
Age (yrs)	37 ± 13	33	[24 - 63]	42 ± 9	43	[24 - 53]
Body mass index (BMI< kg/m2)	26 ± 3	27	[22 - 30]	36 ± 5	36	[30 - 44]
Body weight (kg)	76 ± 9	77	[57 - 89]	104 ± 14	101	[84 - 131]
Total body fat mass (kg)	21 ± 6	20	[13-32]	44 ± 15	46	[23-64]
Total body lean mass (kg)	51 ± 11	48	[40-71]	43 ± 14	46	[23-64]
Trunk lean mass (kg)	26 ± 6	24	[19-39]	27 ± 7	23	[21-41]
Appendicular mass (kg)	23 ± 6	21	[17-33]	26 ± 4	26	[20-34]
Baseline, Low-oxalate diet						
Plasma oxalate (µmol/L)			[<0.5 - 0.67]			[<0.5 - 0.64]
Plasma glycolate (µmol/L)	4.3 ± 0.5	4.5	[3.5 - 4.9]	5.2 ± 1	5.2	[3.8 - 6.8]
Plasma ascorbic acid (µmol/L)	60 ± 13	61	[38 - 81]	52 ± 14	50	[33 - 77]
Renal fraction of excretion of oxalate [a]	1.9 ± 0.4	1.8	[1.4 - 2.5]	1.9 ± 0.6	2	[1,4 - 2.3]
Renal fraction of excretion of glycolate	0.5 ± 0.2	0.5	[0.3 - 0.7]	0.5 ± 0.1	0.4	[0.3-0.8]
Urinary oxalate (mg/day)	19 ± 5	18	[12 - 28]	21 ± 4	21	[14 - 26]
Urinary oxalate (mg/g creatinine)	13 ± 4	12	[8 - 20]	13 ± 3	13	[9 - 17]
Urinary glycolate (mg/day)	29 ± 12	29	[11 - 48]	36 ± 9	38	[21 - 47]
Urinary glycolate (mg/g creatinine)	19 ± 10	15	[10 - 42]	23 ± 8	22	[13 - 38]
Urinary creatinine (g/day)	1.6 ± 0.5	1.5	[1.0-2.4]	1.7 ± 0.5	2.7	[0.9-2.6]
Oxalate Infusion day						
Mean hourly 13C2-oxalate recovery (%)	**99 ± 7**	99	[83 - 109]	91 ± 11	91.8	[72.5 - 106]
Mean urinary 13C2-oxalate MPE (%)	18 ± 5	17	[13 - 27]	21 ± 4	20	[17 - 29]
Mean plasma 13C2-oxalate MPE (%) [a]	18 ± 3	16	[16-22]	18		
Urinary oxalate (mg/hr), T60-T360	0.70 ± 0.2	0.64	[0.51-1.04]	0.71 ± 0.16	0.70	[0.48 - 1.03]
Urinary oxalate (mg/g creatinine) T60-T360	11 ± 3	10	[8-18]	9 ± 1	9	[7 - 11]
Ra oxalate (umol/hr/kg)	0.098 ± 0.03	0.092	[0.072 - 0.156]	0.08 ± 0.02	0.075	[0.060 - 0.122]
Endogenous oxalate synthesis (mg/day)	**16 ± 4**	**15**	**[11 - 23]**	**18 ± 3**	**18**	**[13- 24]**
Estimated 24-hr oxalate synthesis (mg/day), with hourly fasting urines	17 ± 5	15	[12 - 25]	17 ± 4	17	[11 - 25]
Endogenous oxalate synthesis (mg/day/kg lean mass)	0.317 ± 0.09	0.288	[0.245 - 0.555]	0.313 ± 0.04	0.301	[0.245 - 0.374]
Endogenous oxalate synthesis (mg/day/g creatinine)	11 ± 4	9	[8 - 20]	11 ± 2	11	[7 - 16]

The two groups are defined by BMI (< or > 30 kg/m^2), M: male, F: female, AA: African American/Black, C: Caucasian/White, H: Hispanic or Latino, Urinary oxalate and glycolate excretions were calculated as the mean of two 24-hr urine collections under controlled diet. Mean hourly oxalate recovery and urinary MPE were calculated during the last 3-4 steady-state hourly periods and estimated 24-hr oxalate synthesis using mean hourly oxalate excretion at steady state. For adjusted endogenous oxalate synthesis rates, the mean of the two 24-hr urinary creatinine excretion on the controlled diet was used, body composition was measured by DXA-scan or bio-impedance.

Supplemental Table S13. Individual data.

Participant ID	OXI_1	OXI_2	OXI_3	OXI_4	OXI_5	OXI_6	OXI_7	OXI_8	OXI_9	OXI_10	OXI_11	OXI_12	OXI_13	OXI_14	OXI_15	OXI_16	OXI_17	OXI_18	OXI_19	OXI_20
ANTHROPOMETRIC MEASURES																				
Gender	F	F	F	F	F	F	F	F	F	F	F	F	M	M	M	M	M	M	M	M
Age (years)	28.6	47.3	32.9	63.0	35.0	41.7	53.2	44.2	24.2	53.1	44.6	43.5	28.7	54.4	24.8	33.3	23.8	33.9	40.1	37.7
Oxalobacter colonization status	unknown	positive	negative	positive	negative	positive	positive	positive	positive	unknown	unknown	negative	negative	positive	negative	positive	negative	positive	negative	positive
BMI (kg/m2)	21.6	25.1	26.6	27.8	27.8	30.1	35.8	36.2	37.8	38.8	43.9	44.0	23.4	23.9	27.8	28.0	29.5	30.4	31.2	31.9
Waist to Hip ratio	0.75	0.79	0.75	0.96	0.80	0.84	0.82	0.96	0.84	0.86	0.91	0.90	0.84	0.97	0.86	0.93	0.94	0.83	0.95	1.00
Height (cm)	163.0	167.5	160.0	162.8	165.0	167.1	176.7	166.0	165.0	160.6	166.9	172.3	185.2	181.7	179.1	170.0	171.0	184.0	171.0	173.0
Weight (kg)	57.3	70.5	68.0	72.3	75.6	84.1	111.7	99.8	102.8	100.0	122.3	130.7	81.1	78.8	89.3	80.9	86.3	103.0	90.7	95.4
Fat Mass (%, TANITA)	26.3	28.3	34.0	38.2	33.8	43.7	46.3	45.7	43.7	49.4	65.2	49.8	10.9	11.6	16.7	27.6	24.3	22.5	27.0	28.4
Total Body Water (kg, TANITA)	30.90	37.0	32.7	32.7	36.7	34.7	43.9	36.3	42.3	37.1	41.8	48.1	52.2	49.2	54.5	42.9	47.8	57.4	49.1	50.0
Fat Free Mass (kg, TANITA)	42.20	50.5	44.6	44.7	50.1	47.3	59.9	49.6	57.8	50.6	57.2	65.6	71.4	67.2	74.4	58.6	65.3	78.4	67.0	68.3
Fat Mass (kg, TANITA)	15.1	20.0	23.1	27.6	25.6	36.8	51.8	45.6	45.0	49.4	53.3	65.1	8.7	14.7	14.9	22.3	21.0	23.2	24.5	27.1
Predicted Trunk Lean Mass (kg, TANITA)	23.0	28.2	23.7	24.1	27.5	25.4	31.6	27.8	31.1	27.0	29.6	34.6	37.9	35.4	38.5	29.7	33.3	40.7	34.6	35.2
Predicted Appendicular Mass (kg, TANITA)	17.1	19.8	17.7	17.5	20.1	19.6	25.2	21.4	23.8	20.9	24.7	27.7	30.2	27.4	32.6	26.2	29.1	34.1	29.4	30.0
Predicted Lean Mass (kg, TANITA)	40.1	48.0	41.4	41.6	47.6	45.0	56.8	49.2	54.9	47.9	54.3	62.3	68.1	62.8	71.1	55.9	62.4	74.8	64.0	65.2
Body mass (kg, DXA)	56.2	70.9	MS	70.5	MS	85.1	108.9	MS	100.1	99.9	120.1	129.0	81.4	76.8	MS	82.3	MS	MS	MS	MS
Total Bone Mineral content (g, DXA)	2646	2753	MS	2332	MS	2455	2881	MS	2552	2844	3090	3411	3599	3617	MS	2586	MS	MS	MS	MS
Relative Skeletal Muscle Index (kg/m2, DXA)	6.83	7.63	MS	6.57	MS	7.05	8.64	MS	9.19	9.58	9.98	10.91	8.96	7.64	MS	7.42	MS	MS	MS	MS
Visceral Adipose Tissue (g, DXA)	143	416	MS	1074	MS	709	1419	MS	794	1305	1482	1893	386	421	MS	1041	MS	MS	MS	MS
Fat Mass (kg, DXA)	13.17	22.56	MS	28.07	MS	39.20	55.78	MS	45.76	47.34	63.75	58.61	15.72	19.34	MS	32.00	MS	MS	MS	MS
Trunk Lean Mass (kg, DXA)	19.18	21.27	MS	19.41	MS	21.00	20.75	MS	22.69	21.44	21.85	21.44	27.72	24.68	MS	22.46	MS	MS	MS	MS
Appendicular Mass (kg, DXA)	18.06	21.2	MS	17.4	MS	19.5	25.8	MS	25.8	24.5	28.0	24.5	30.6	25.2	MS	21.5	MS	MS	MS	MS
Lean Mass (kg, DXA)	40.36	45.57	MS	40.14	MS	43.43	50.26	MS	51.78	49.70	53.26	66.97	62.09	53.83	MS	47.76	MS	MS	MS	MS
Fat Mass (kg, mix)	13.17	22.56	18.70	28.07	25.50	39.20	55.78	46.00	45.76	47.34	63.75	58.61	15.72	19.34	14.90	32.00	21.00	22.80	24.80	27.10
Trunk Lean Mass (kg, mix)	19.18	21.27	23.7	19.41	27.5	21.00	20.75	27.8	22.69	21.44	21.85	21.44	27.72	24.68	38.5	22.46	33.3	40.7	34.6	35.2
Appendicular Mass (kg, mix)	18.06	21.25	17.70	17.37	20.10	19.51	25.77	21.40	25.84	24.53	28.04	24.53	30.63	25.21	32.60	21.48	29.10	34.10	29.40	30.00
Lean Muscle Mass (kg, mix)	40.36	45.57	41.40	40.14	47.60	43.43	50.26	49.20	51.78	49.70	53.26	66.97	62.09	53.83	71.10	47.76	62.40	74.80	64.00	65.20
Fixed low-oxalate diet kCal/day	2000	2000	2000	2000	2000	2000	2000	2500	2000	2000	2000	2500	2500	2500	2500	2500	2500	2500	2500	2500
BLOOD VALUES - MEASURED																				
Plasma Creatinine (mg/dl)	0.60	0.8	0.7	0.9	0.7	0.6	0.7	0.8	0.8	0.8	1.1	0.9	1.0	1.0	0.8	1.0	1.1	0.9	1.2	0.7
Plasma Ascorbic acid (uM)	57	81	68	50	71	27	MS	59	40	49	77	33	38	64	56	50	69	47	64	56
Plasma Oxalate (uM)	0.47	0.34	0.54	0.45	0.53	0.36	0.38	0.38	0.56	0.34	0.34	0.42	0.55	0.67	0.49	0.52	0.31	0.35	0.64	0.41
Plasma Glycolate (uM)	4.9	3.5	4.9	4.5	4.5	5.1	6.8	4.8	4.7	5.7	5.3	6.3	4.0	3.8	4.5	4.0	4.7	4.2	5.7	3.8
BLOOD VALUES - CALCULATED																				
eGFR (ml/mn)	120	91	117	72	116	115	103	93	105	88	81	81	105	89	126	102	96	115	78	121
Fraction of Excretion of Oxalate	NA	NA	2.52	NA	1.38	NA	NA	NA	1.44	NA	NA	NA	1.72	1.80	1.84	2.10	NA	NA	2.31	NA
Fraction of Excretion of Glycolate	MS	0.64	0.65	0.31	0.69	0.65	0.41	0.79	0.61	0.34	0.42	0.36	0.26	0.43	0.40	0.70	0.47	0.36	0.45	0.46
INFUSION PARAMETERS - MEASURED																				
13C2-Oxalate Infusion rate (ml/hr)	89	109	136	112	133	131	87	143	80	77	95	102	125	123	113	126	143	155	143	123
Hours of Infusion (hr)	6	6	5	6	5	6	6	5	6	6	6	6	6	6	6	6	5	5	5	6
Infusion Bolus (ml)	175	240	200	220	195	192	192	210	176	151	187	224	245	241	247	227	210	227	210	181
Total Infusion volume (ml)	709	894	880	892	860	978	714	925	656	613	757	836	995	979	925	983	925	1002	925	919
13C2-oxalate infusion concentration (uM)	10.46	10.6	16.0	9.8	16.2	19.0	20.0	20.4	19.5	20.3	20.6	18.8	10.3	10.3	23.1	9.9	17.3	18.3	18.0	22.4
INFUSION PARAMETERS - CALCULATED																				
Total 13C2-oxalate infused (mg)	0.683	0.869	1.295	0.807	1.280	1.711	1.313	1.739	1.177	1.145	1.433	1.447	0.946	0.924	1.964	0.898	1.473	1.687	1.534	1.893
13C2-oxalate infusion rate (nmol/hr/kg)	16.3	16.3	32.0	15.2	28.5	29.6	15.6	29.3	15.2	15.6	16.0	14.7	15.9	16.0	29.2	15.5	28.7	27.5	28.4	28.9
URINE VALUES - MEASURED																				
Mean 24-hr Urinary Creatinine (g/day on controlled diet)	1.02	1.210	1.152	1.085	1.550	1.207	0.909	2.005	1.139	1.517	1.710	1.632	2.033	1.677	2.440	1.543	2.051	2.581	2.242	2.175
Mean 24-hr Urinary Oxalate (mg/day on controlled diet)	15.51	15.60	22.90	16.12	12.47	20.96	14.42	18.81	18.48	18.76	22.38	24.37	17.70	22.97	28.12	17.38	19.86	22.31	26.33	21.23
Mean 24-hr Urinary Oxalate (mg/day/g creatinine on controlled diet)	15.15	12.88	19.87	14.86	8.04	17.18	15.86	9.42	16.14	12.39	13.09	14.94	8.70	13.72	11.43	11.27	9.96	9.08	11.74	9.74
Mean 24-hr Urinary Glycolate (mg/day on controlled diet)	11.02	28.30	48.22	21.36	15.30	35.37	21.45	28.32	43.68	37.82	21.70	46.41	30.27	24.52	32.46	47.12	31.51	37.46	47.08	38.71
Mean 24-hr Urinary Glycolate (mg/day/g creatinine on controlled diet)	10.77	23.44	42.09	19.64	9.87	29.42	23.59	14.25	37.78	25.03	12.68	28.43	14.89	14.59	13.42	30.37	15.40	15.28	21.00	17.76
Excess Mole Percent Enrichment of 13C2-oxalate in urine at steady-state (%)	14.86	18.16	16.82	15.25	27.09	29.48	20.35	26.73	18.62	16.76	18.06	17.25	13.33	12.60	19.92	17.56	23.68	24.95	18.81	22.86
Excess Mole Percent Enrichment of 13C2-oxalate in plasma at steady-state (%)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	16%	NA	22%	16%	NA	NA	18%	NA
Mean urinary oxalate excretion during infusion (mg/hr, T60-T360)	0.51	0.51	1.04	0.58	0.53	0.48	0.67	0.68	0.61	0.56	0.77	0.76	0.76	0.68	1.03	0.60	0.77	0.71	1.03	0.85
Mean urinary oxalate excretion during infusion (mg/g creatinine, T60-T360)	10.46	9.60	17.60	11.76	8.35	8.74	10.24	8.30	9.49	8.43	10.90	9.33	9.27	11.08	9.50	10.01	7.55	6.73	10.82	9.59
Total urinary oxalate excretion on day of infusion (mg/24-hr)	14.60	9.64	19.87	16.96	14.45	18.29	15.01	18.45	19.17	16.75	21.22	13.22	20.39	20.70	33.21	14.54	23.83	21.18	28.85	22.48
Total urinary oxalate excretion on day of infusion (mg/g creatinine)	12.69	10.59	19.04	14.37	9.47	13.96	10.03	9.46	14.49	11.49	11.67	11.63	9.94	13.37	12.00	11.01	10.02	8.51	13.16	10.93
URINE VALUES - CALCULATED																				
Total 13C2-oxalate tracer recovery in urine over 24-hr	94%	104%	97%	100%	101%	78%	98%	83%	84%	76%	93%	78%	97%	88%	104%	102%	108%	88%	80%	90%
Mean 13C2-oxalate tracer recovery in urine at steady-state	100%	103%	97%	108.5%	98%	76.7%	102.4%	97%	94%	72.5%	91.6%	86.1%	99.2%	82.7%	105%	101.8%	99%	92%	87%	106%
Endogenous Oxalate Synthesis (mg/day)	11.48	11.16	22.97	13.16	12.44	12.80	14.63	17.21	14.66	16.70	19.05	19.78	18.06	18.82	22.53	12.63	17.14	18.33	23.91	19.97
Estimated 24-hr urinary oxalate synthesis using hourly fasting urine excretion (mg/day)	12.14	12.29	24.87	13.84	12.78	11.49	16.17	16.30	14.67	13.47	18.50	18.15	18.35	16.29	24.67	14.47	18.37	17.09	24.82	20.33
Rate of appearance of oxalate (umol/hr/kg body weight)	0.093	0.073	0.156	0.084	0.076	0.070	0.061	0.080	0.066	0.077	0.072	0.070	0.103	0.111	0.117	0.072	0.092	0.082	0.122	0.097
Endogenous Oxalate Synthesis (mg/day/g creatinine)	11.21	9.22	19.94	12.13	8.02	10.60	16.09	8.59	12.87	11.01	11.14	12.12	8.88	11.22	9.23	8.18	8.36	7.10	10.66	9.18
Endogenous Oxalate Synthesis (mg/day/kg lean mass by mixed assessment)	0.284	0.245	0.555	0.328	0.261	0.295	0.291	0.350	0.283	0.336	0.358	0.295	0.291	0.350	0.317	0.264	0.275	0.245	0.374	0.306
Endogenous Oxalate Synthesis (mg/day/kg lean mass by TANITA)	0.286	0.232	0.555	0.316	0.261	0.284	0.258	0.350	0.267	0.349	0.351	0.318	0.265	0.300	0.317	0.226	0.275	0.245	0.374	0.306
Endogenous Oxalate Synthesis (mg/day/kg lean mass by DXA)	0.284	0.245	MS	0.328	MS	0.295	0.291	MS	0.283	0.336	0.358	0.295	0.291	0.350	MS	0.264	MS	MS	MS	MS
Endogenous Oxalate Synthesis (mg/day/kg appendicular mass by mixed assessment)	0.621	0.434	1.127	0.699	0.399	0.543	0.624	0.401	0.498	0.449	0.397	0.494	0.290	0.445	0.283	0.381	0.287	0.208	0.363	0.306
Endogenous Oxalate Synthesis (mg/day/kg appendicular mass by TANITA)	0.656	0.466	1.127	0.693	0.399	0.541	0.639	0.401	0.541	0.527	0.451	0.438	0.294	0.410	0.283	0.312	0.287	0.208	0.363	0.306
Endogenous Oxalate Synthesis (mg/day/kg appendicular mass by DXA)	0.636	0.525	MS	0.758	MS	0.656	0.568	MS	0.567	0.681	0.680	0.806	0.590	0.747	MS	0.588	MS	MS	MS	MS
Endogenous Oxalate Synthesis (mg/day/kg trunk lean mass by mixed assessment)	0.585	0.433	0.841	0.625	0.292	0.505	0.776	0.309	0.567	0.513	0.510	0.565	0.320	0.455	0.240	0.364	0.251	0.174	0.308	0.261
Endogenous Oxalate Synthesis (mg/day/kg trunk lean mass by TANITA)	0.488	0.327	0.841	0.503	0.292	0.417	0.509	0.309	0.414	0.408	0.376	0.350	0.234	0.317	0.240	0.275	0.251	0.174	0.308	0.261
Endogenous Oxalate Synthesis (mg/day/kg trunk lean mass by DXA)	0.585	0.433	MS	0.625	MS	0.505	0.776	MS	0.567	0.513	0.510	0.565	0.320	0.455	MS	0.364	MS	MS	MS	MS

M: male, F: female, AA: African American/Black, C: Caucasian/White, H: Hispanic or Latino, MS: missing data. NA: results < limit of quantification. Results expressed as median [range]. eGFR was calculated using the CKD-EPI creatinine equation (2021) without race adjustment. Plasma was collected fasted, at the end of 4 days of low-oxalate diets (See Sup. Methods for details). Plasma oxalate was below detection level of the method (0.5 µM) in all, but 8 participants. Urinary oxalate and

glycolate excretions were calculated as the mean of two 24-hr urine collection under controlled diet. Mean hourly oxalate recovery and mean urinary MPE were calculated during the last 3-4 steady-state hourly periods. Estimated 24-hr oxalate synthesis was calculated as the pro-rata to 24-hr of the average of 5-6 hourly excretions of oxalate during the infusion (T60-T360), in the fasted state, see Sup. Methods. For adjusted endogenous oxalate synthesis rates, the mean of the two 24-hr urinary creatinine excretion on the controlled diet was used, in order to avoid potential underestimation of creatinine due to multiple collection on the infusion day. Body composition (fat, lean, lean trubk, appendicular mass and bone mineral content) was calculated using the segmental body mass composition by TANITA and by DXA-scan, as indicated, or by mixed assessment (DXA if available and TANITA if not).

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