THE UNIVERSITY OF ALABAMA AT BIRMINGHAM
Heersink School of Medicine

# Oxalobacter formigenes, an oxalate-degrading gut commensal, and urinary oxalate excretion


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# Disclosures

No interests to disclose

# Outline



1. Oxalate Homeostasis
2. Oxalate and the microbiome
3. *Oxalobacter formigenes*
4. Fixed diet human studies and *O. formigenes*
5. Summary

# Our lab – UAB Kidney Stone Research Lab



## Oxalate and Calcium Oxalate Kidney Stone Disease

### Specific Research Areas

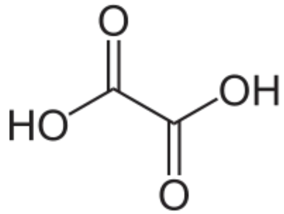
- Endogenous Oxalate Synthesis
- Dietary Oxalate Absorption
- *Oxalobacter formigenes*

# Oxalate



Most acidic dicarboxylic acid

- In vivo, exists as a divalent anion
- Readily complexes with cations (Ca, Mg)




In humans:

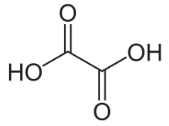
- End product of metabolism
- Derived from gut absorption of dietary oxalate
- Primarily excreted in urine
- Increased amounts of urinary oxalate is a risk factor for calcium oxalate kidney stone formation

Spinach: ~1 g oxalate per 100g

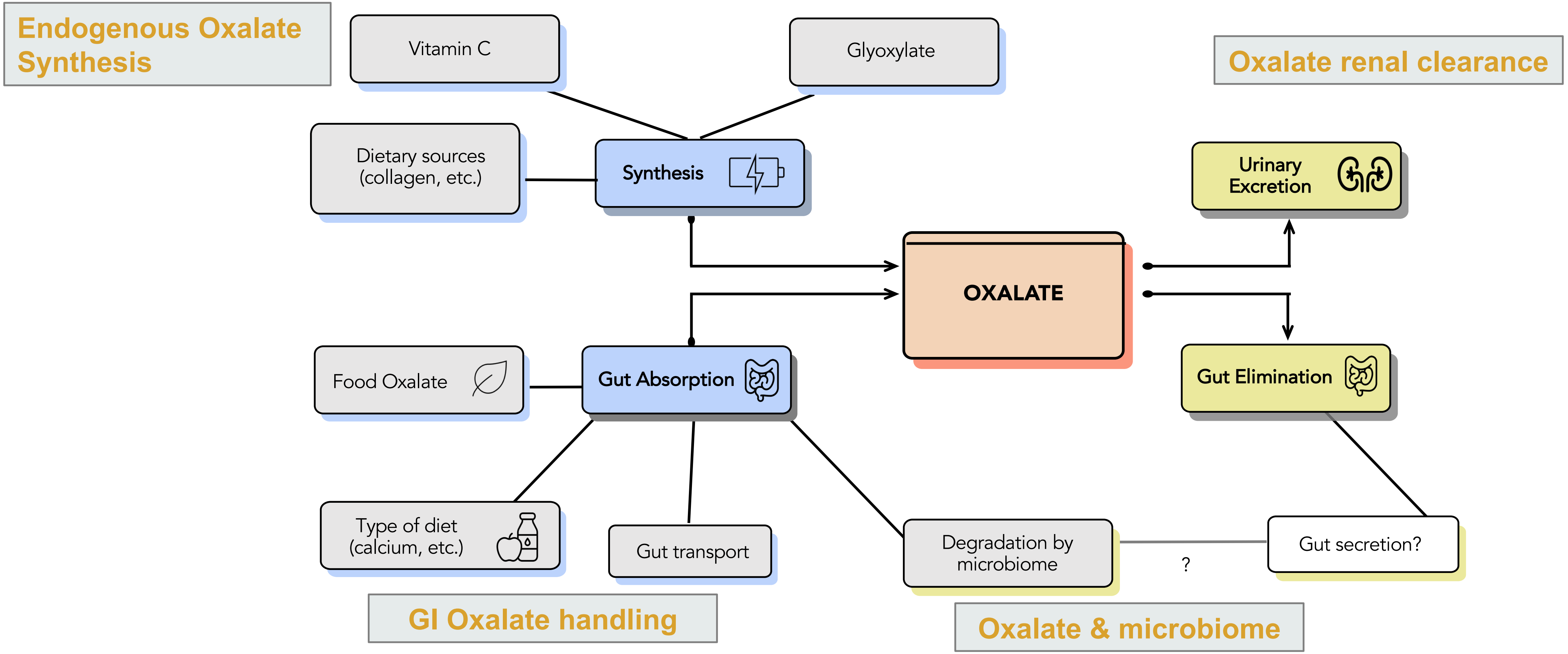
Plants primary source of dietary oxalate intake

Resident gut bacteria degrade oxalate

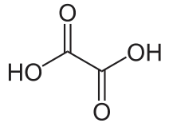
# Oxalate Homeostasis



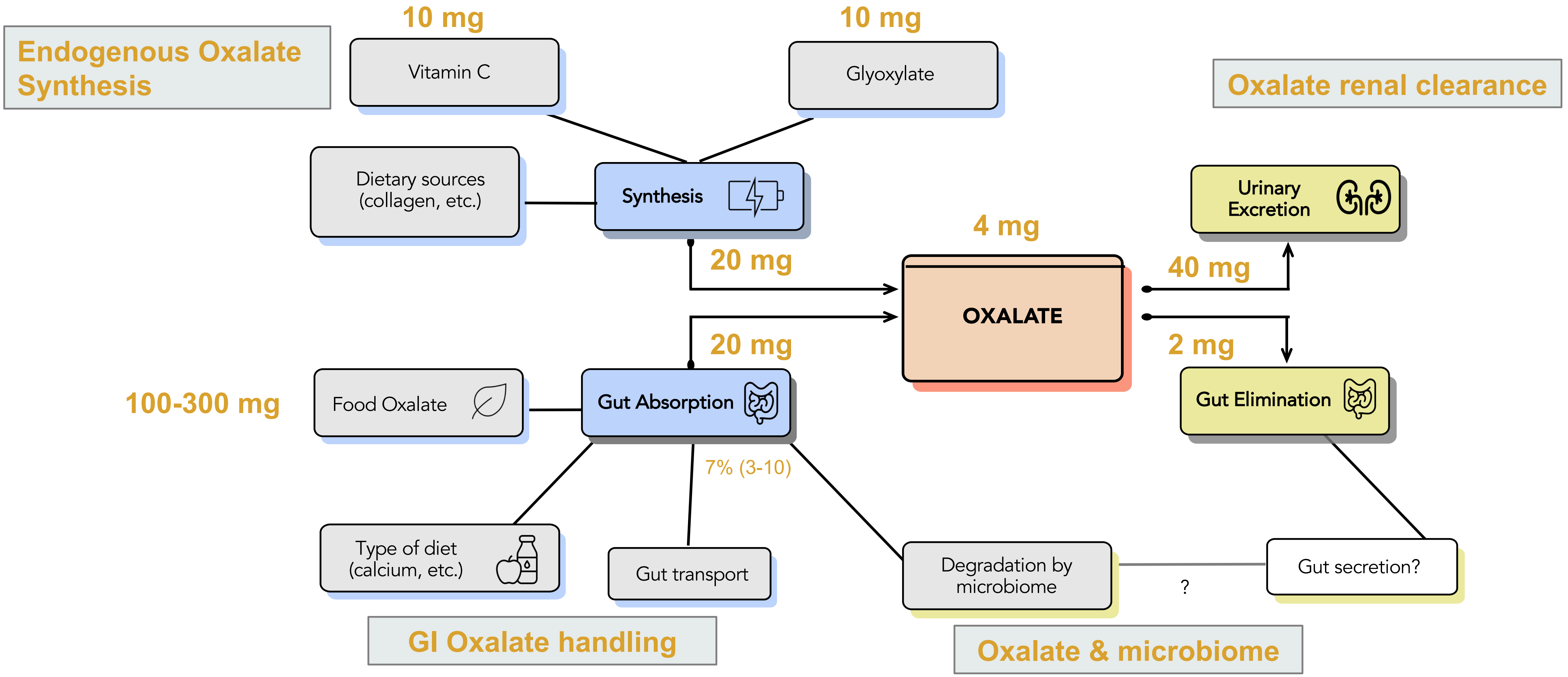


# Oxalate Homeostasis





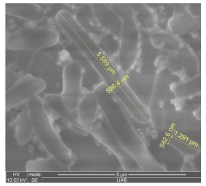
# Oxalate & Microbiome



## Oxalate degrading bacteria

Genes involved in oxalate degradation in numerous commensal gut bacterial species

- Oxalate oxidoreductase
- Oxalate decarboxylase
- Formyl-CoA:oxalate CoA transferase
- Oxalyl-CoA decarboxylase



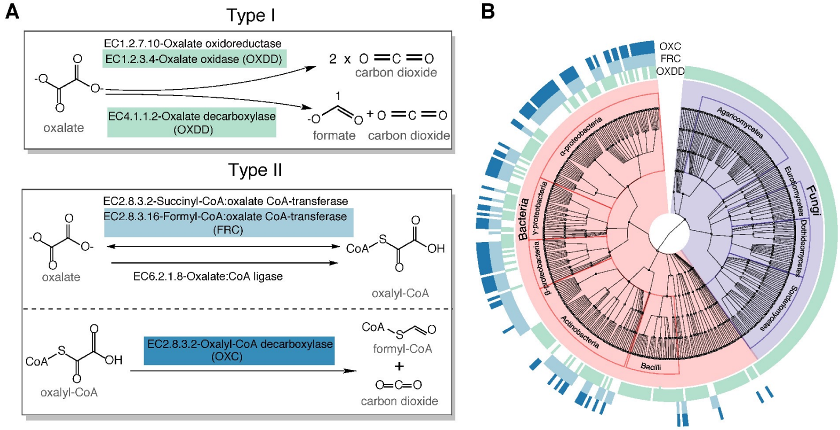
*1940s - 1950s : oxalate degrading microbes in human feces and in the rumen*

*1985: same species of bacteria isolated from sheep rumen isolated in human feces: Oxalobacter formigenes*

Specialist oxalate degrader: *Oxalobacter formigenes*

Generalists oxalotrophs: *E coli, Bifidobacterium spp, Lactobacillus spp, etc.*

Gut oxalate-degrading microbial network




*Liu, Elife 2021*

*Dawson, AEM 1980; Allison, Arch Microbiol 1985; Kaufman, JASN 2008; Liu, Elife 2021; Daniels, AEM 2021; Jiang, J Urol 2011, Ermer Nat Rev Nephrol 2023.*

# Oxalate Degrading Bacteria: Brief Historical Perspective



1940s and 1950s : discovery of oxalate degrading microbes in human feces and in the rumen

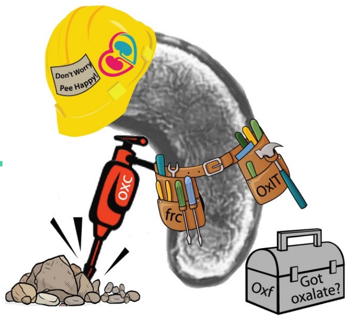
1977: Strictly anaerobic bacteria primarily responsible for oxalate degradation in sheep rumen (Allison MJ, et. al. J Anim Sci 45:1173–1179.)

1980: First anaerobic oxalate degrading bacterium isolated from sheep rumen by Karl Dawson (Dawson KA, et. al. Appl Environ Microbiol 40).

1985: same species of bacteria isolated by Dawson was isolated in human feces by Milton Allison and colleagues and given the name Oxalobacter formigenes (Allison MJ, et. al.. Arch Microbiol 141)

Milton Allison, PhD
Iowa State University

# *Oxalobacter formigenes*

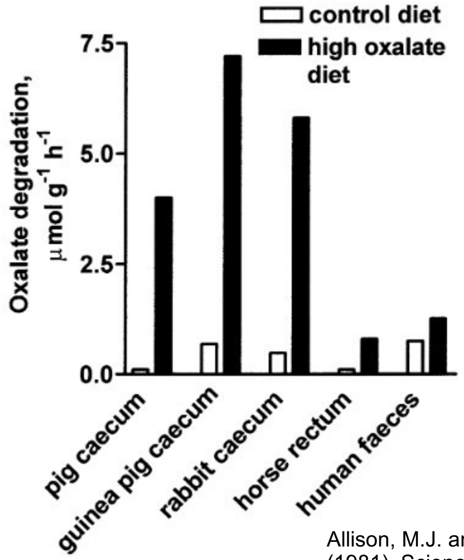



Gram-negative strict obligate anaerobe colonic bacterium

Uses only oxalate as an energy source and converts it to formate and CO2

Increasing the amount of oxalate in the diet increases the rate of gut microbial oxalate degradation

O.formigenes primarily responsible for oxalate breakdown in gut (colon)

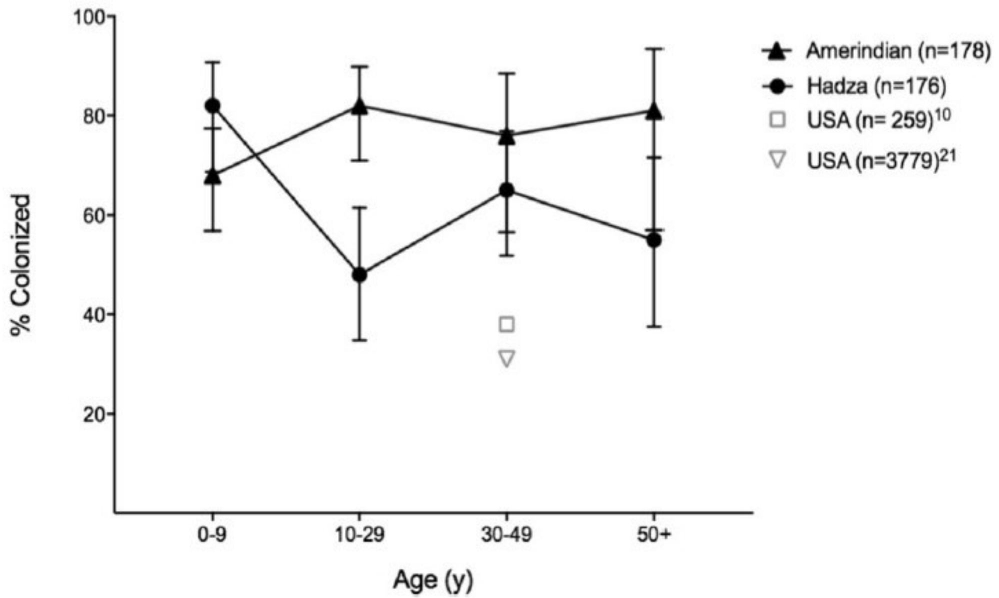



Allison, M.J. and Cook, H.M. (1981). Science 212

# Prevalence of *O.formigenes* in human populations

Lower colonization in US adult cohort

- Higher Antibiotic exposure?




PeBenito, A., Nazzal, L., Wang, C. *et al.* *Sci Rep* **9,** 574 (2019)

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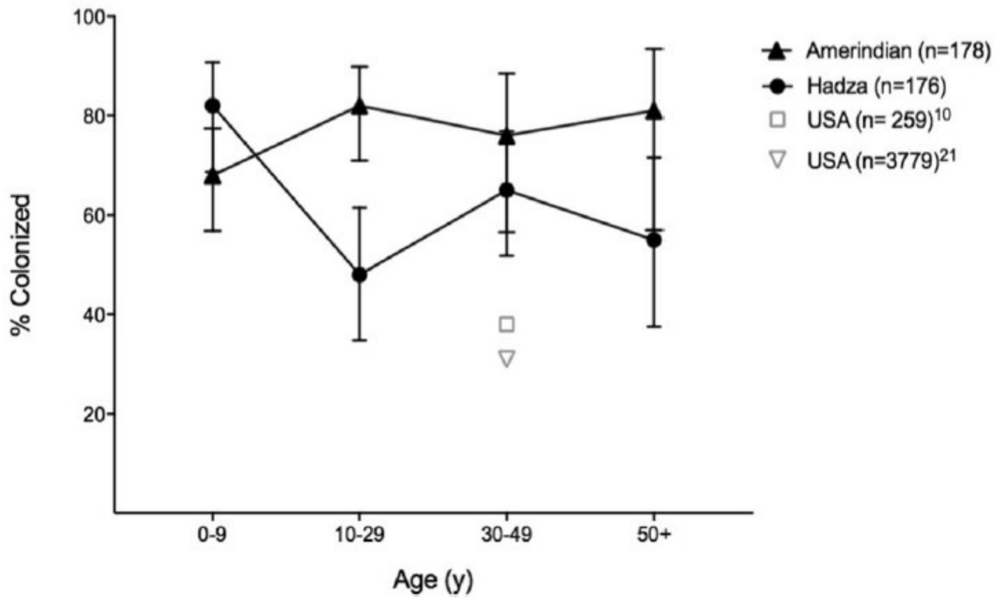
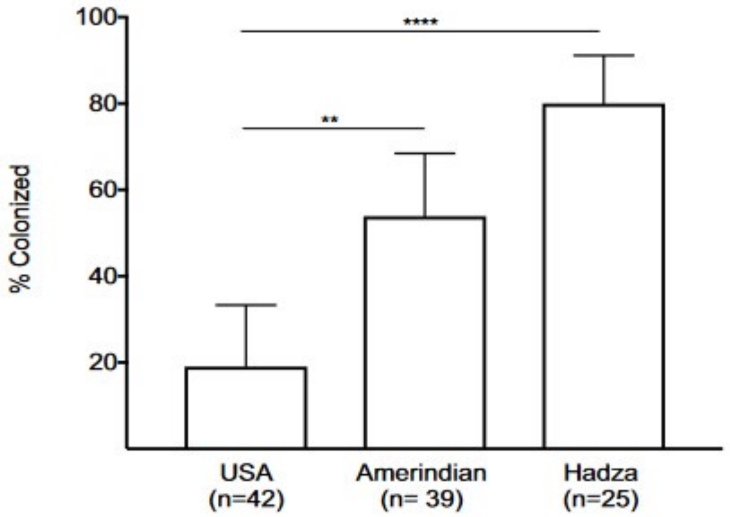



PeBenito, A., Nazzal, L., Wang, C. *et al. Sci Rep* **9,** 574 (2019)

In children < 5 years of age

No evidence of colonization in USA children until 12 months of age.

Hadza and Amerindian populations: colonization earliest at 9 months and 3 months of age, respectively

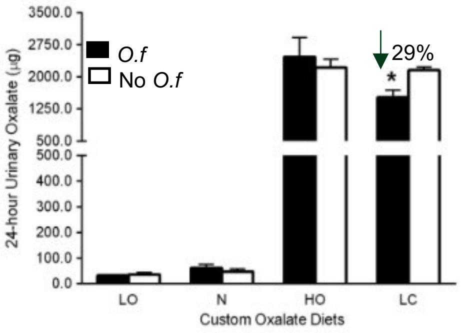


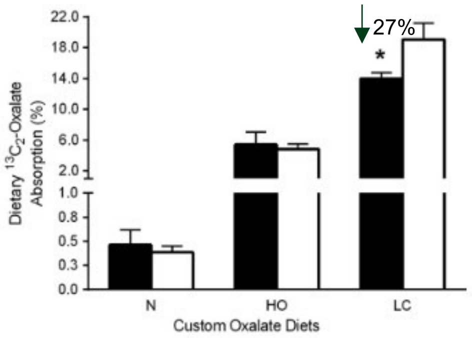

PeBenito, A., Nazzal, L., Wang, C. *et al. Sci Rep* **9,** 574 (2019)

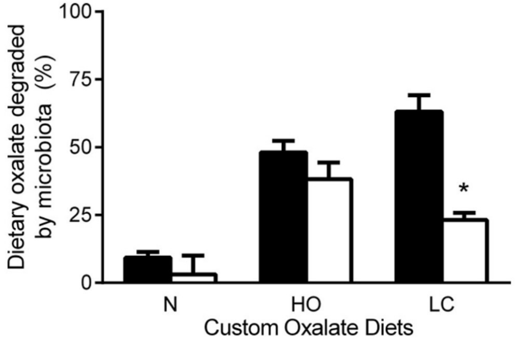
# Rodent Studies: Colonization and Urinary Oxalate



Studies with rats/mice have shown a decrease in urinary oxalate excretion with *O.formigenes* colonization




| Mouse Diets | Calcium : Oxalate Mole Ratio | Oxalate (mg/g diet) | Calcium (mg/g diet) |
|---|---|---|---|
| LO | - | 0.010 | 5 |
| N | 11.3 | 1 | 5 |
| HO | 0.8 | 15 | 5 |
| LC | 0.2 | 2.5 | 0.25 |

Li X, Ellis ML, Knight J. Oxalobacter formigenes Colonization and Oxalate Dynamics in a Mouse Model. *Appl Environ Microbiol*. 2015;81(15):5048-5054. doi:10.1128/AEM.01313-15

# *O.formigenes* Colonization & Idiopathic CaOx Stone Disease



*O.formigenes* colonization rate is reduced in CaOx kidney stone formers

Largest US study to date1 (247 CaOx KSF and 259 controls)

37% of controls were colonized with *O.formigenes*,

only 17% of Kidney Stone Formers were colonized

Odds ratio for forming a recurrent CaOx stone was 0.3 [95% CI: 0.2-0.5] in colonized vs non-colonized individuals

**BUT**

Effects on urinary oxalate uncertain due to

lack of controlled diet studies

targeting of appropriate population (dietary oxalate)

*Parsells KellyJ. et al. Factors related to colonization with Oxalobacter formigenes in U.S. adultsJ Endourol. 2011 Apr;25(4):673-9*

*Kaufman, D. W. et al. Oxalobacter formigenes may reduce the risk of calcium oxalate kidney stones. J. Am. Soc. Nephrol 19, 1197–1203 (2008).*

# *O.formigenes* & Urinary Oxalate - Fixed Diet Studies



Controlled diet study in naturally colonized and non-colonized healthy non-stone formers (completed in 2011)

Controlled diet sequential study in non colonized healthy non-stone formers – inducing colonization (completed, submitted for publication)

Controlled diet sequential study in non colonized CaOx kidney stone formers – inducing colonization (recruiting, NIDDK R01 funding)

# *O. formigenes* - Natural colonization study

**Study participants**

| | |
|---|---|
| *O. formigenes* colonized | N = 11 (4M/7F) |
| *O. formigenes* non colonized | N = 11 (6M/5F) |
| (age 30 ± 5 years) | |

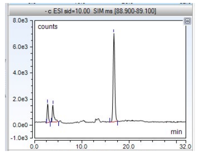
**Fixed Diet Study** Varying oxalate (50 - 750 mg/d), Varying calcium (400-2000 mg/d)
Two 3-week dietary phases, wash-out, completed within 7 to 9 weeks

Dietary Calcium 1000mg
Vary Dietary oxalate

Dietary oxalate 250 mg
Vary Dietary calcium

| 50 mg | 250 mg | 750 mg | 400mg | 2000 mg |
|---|---|---|---|---|
| 7 days | 7 days | 7 days | 7 days | 7 days |

**Outcome**
24-hr urine and stool collected on days 4-7 (3 days equilibration)
Fecal O.formigenes # [qPCR]
Fecal oxalate by Ion Chromatography coupled with Mass spectrometry (IC-MS, all stool homogenized with HCL)
24-hr Urinary Oxalate measured by IC-MS



Oxalate Mass Chromatogram

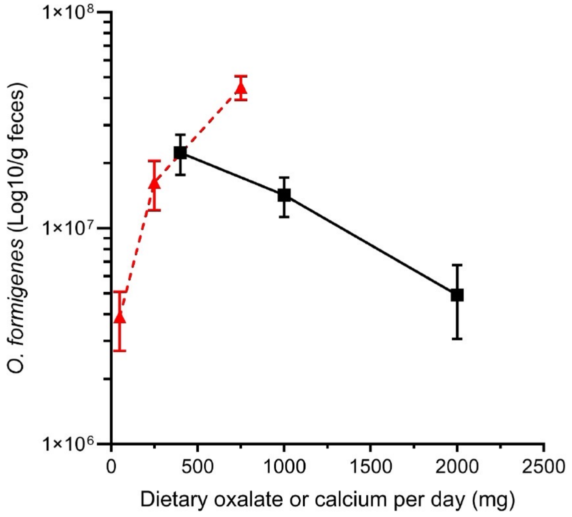
*Jiang et. al. Journal of Urology 2011, vol 186; 135 - 139*

# *O. formigenes* - Natural colonization study



**Results**

Fecal O.formigenes Numbers




Varying Dietary Oxalate

Varying Dietary Calcium

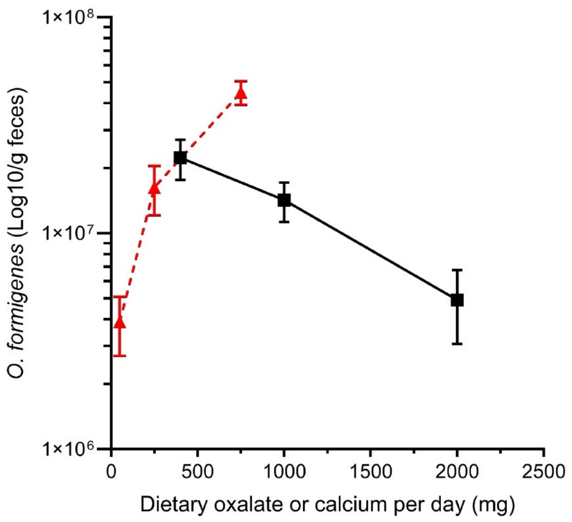
7/11 Group I strain
4/11 Group II strain

*Jiang et. al. Journal of Urology 2011, vol 186; 135 - 139*

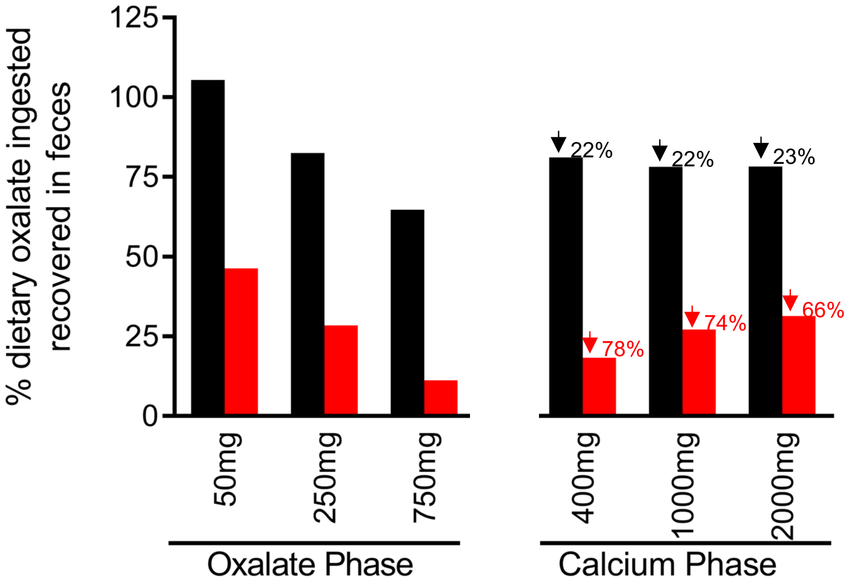
# *O. formigenes* - Natural colonization study

**Results**

Fecal O.formigenes Numbers




Oxalate Degrading Function of Gut Microbiota



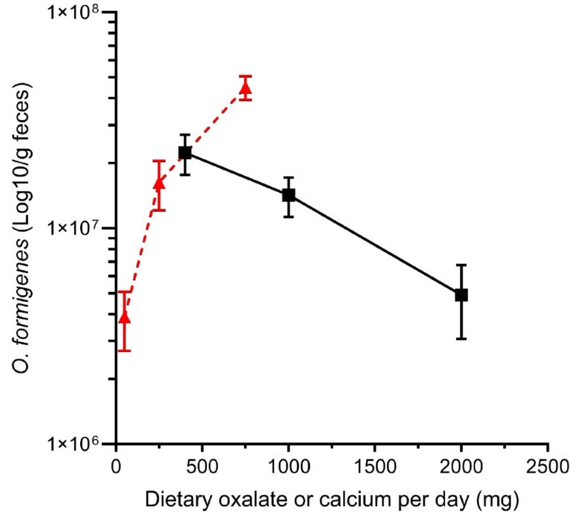

*Jiang et. al. Journal of Urology 2011, vol 186; 135 - 139*

# *O. formigenes* - Natural colonization study



## Results

### Fecal O.formigenes Numbers

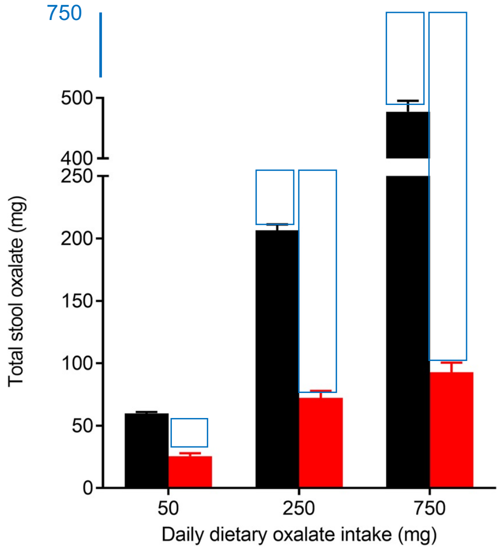



Varying Dietary Oxalate

Varying Dietary Calcium

### Oxalate Degrading Function of Gut Microbiota

*Greater oxalate-degrading capacity in O.f colonized volunteers*




*Stool oxalate in non-colonized and O.f colonized healthy volunteers on fixed Ca (1000 mg/d) & varying oxalate diets*

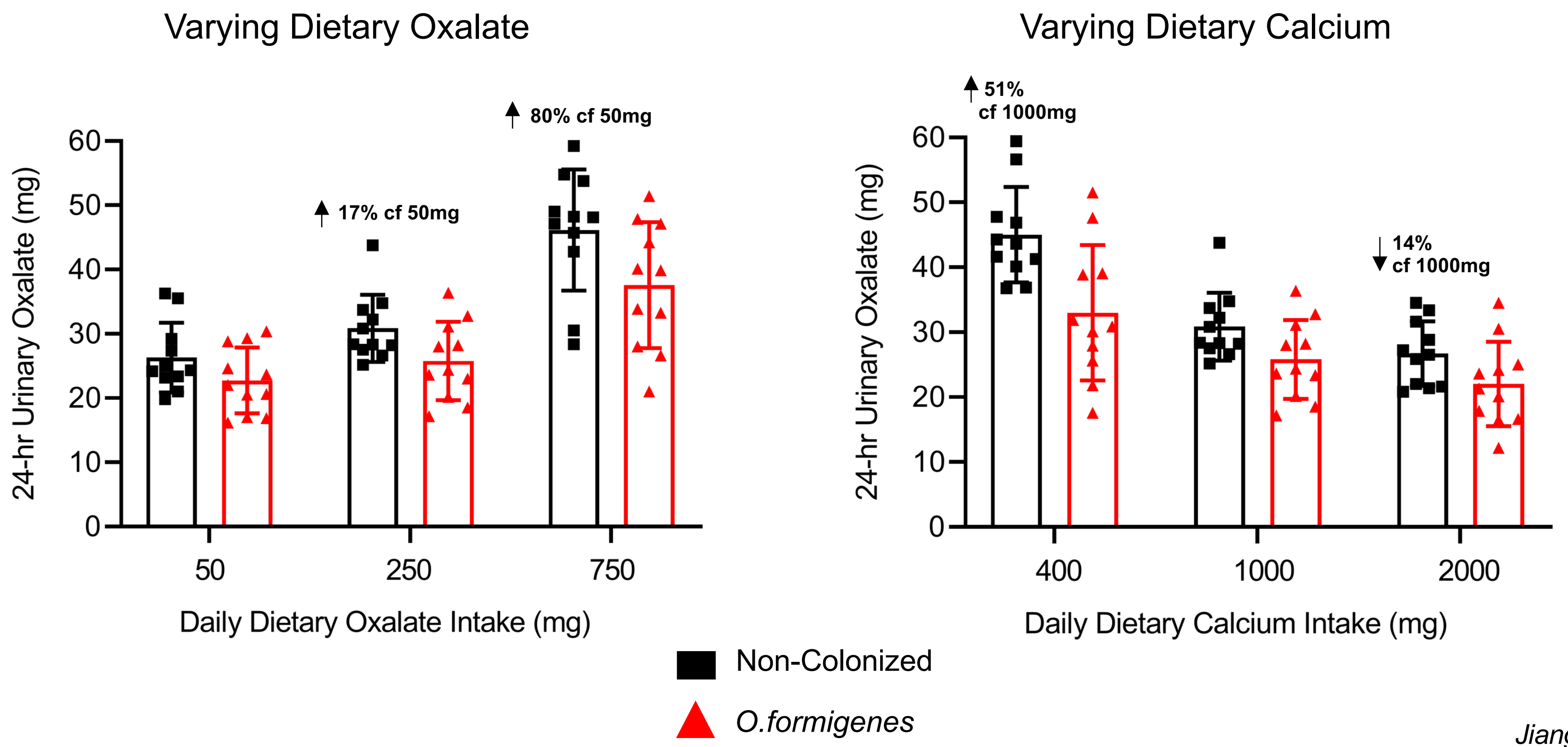
*Jiang et. al. Journal of Urology 2011, vol 186; 135 - 139*

# *O. formigenes* - Natural colonization study

**Results**

24 hour Urinary Oxalate Excretion

significant differences in oxalate excretion only on the 400 mg calcium diet (p = 0.031).




*Jiang et. al. Journal of Urology 2011, vol 186; 135 - 139*

# Conclusion



**Central role** of *O. formigenes*

*O.formigenes* is at the center of the intestinal oxalate-degrading microbial network in humans

*O.formigenes* colonization reduces urinary oxalate excretion during periods of low calcium intake

# *O. formigenes* - Inducing colonization study

**Study participants**

Healthy Volunteers — N = 23 (12M/11F), age 21- 61 years

**O.Formigenes Administration**

Single dose live O.f cells
oxalate rich meal + bicarbonate

**Outcome**

Colonization rate and sustainability of colonization

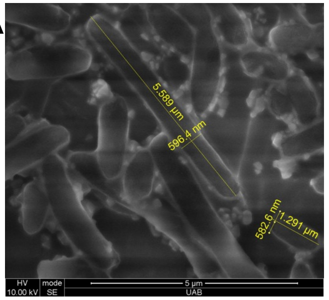
*NCT03752684, submitted*

# *O.formigenes* Colonization Procedure



Ingestion of live *O. formigenes* cells (frozen glycerol paste)
With sandwich

A




~$10^{10}$ *O.formigenes (Human isolate OxCC13)*

B

Spinach Pesto Turkey Sandwich (2mmoles oxalate)

C


Colonization tested 1-4 weeks later by fecal culture

# *O. formigenes* - Inducing colonization study

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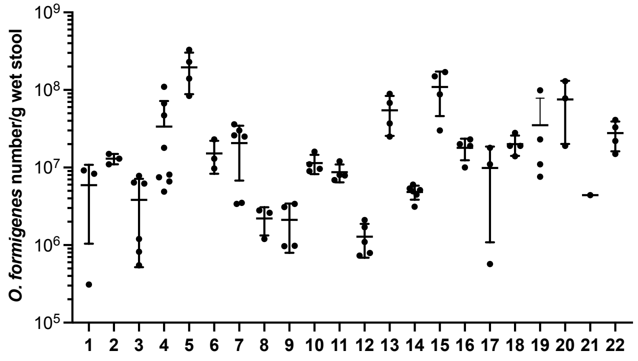
**O.Formigenes Administration**

Single dose live O.f cells
oxalate rich meal + bicarbonate

**Results**

Successful colonization in all subjects
Sustained colonization over 1 year in 10 HV (up to 3 yrs)
Colonization lost in 11, of which 9 reported antibiotic use
6 HV redosed and 5 successfully recolonized




*O.F enumeration in stool (qPCR)*

*NCT03752684, submitted*

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Successful colonization in all subjects
Sustained colonization over 1 year in 10 HV (up to 3 yrs)
Colonization lost in 11, of which 9 reported antibiotic use
6 HV redosed and 5 successfully recolonized

**Study participants**

Individuals with Kidney Stones — N = 5
Individuals with RYGB — N = 4

**O.Formigenes Administration** — Single dose live O.f cells, simple sandwich

Successful colonization in all subjects
Sustained colonization over 1 year in 1 RYGB

*NCT03752684, submitted*

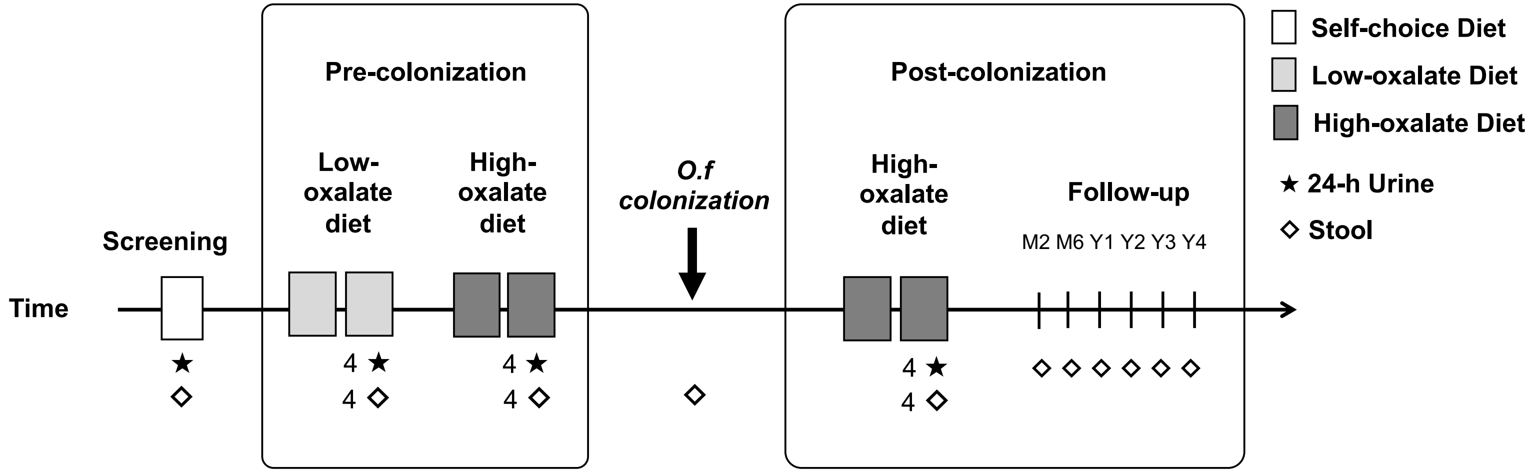
# *O. formigenes* - Inducing colonization study

**Study participants**

Healthy Volunteers N = 22 (12M/10F), age 33 ± 9 yrs

**Fixed Diet Study**

Sequential study , before / after inducing colonization
Moderately high oxalate (250 mg/d), moderately low calcium diet (800 mg/d)




**Outcome**

Stool *O.formigenes* number (qPCR)
stool oxalate content (IC-MS), days 3-5, after 2 days dietary equilibration
24-hr urine oxalate excretion (IC-MS), days 3-5, after 2 days dietary equilibration

*NCT03752684, submitted*

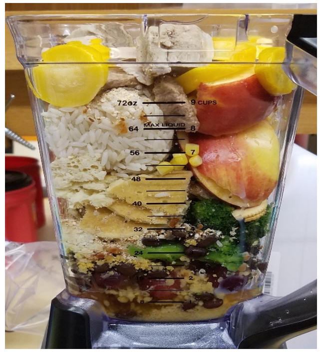
# Controlled Diets



| Diets | % Kcal CHO | % Kcal Fat | % Kcal Protein | Oxalate (mg) | Calcium (mg) |
|---|---|---|---|---|---|
| Low Oxalate | 51 ± 3 | 30 ± 1 | 19 ± 2 | 36 ± 4 (33-41) | 1204 ± 167 |
| Low Calcium | 53 ± 3 | 30 ± 1 | 18 ± 2 | 253 ± 23 (234-279) | 444 ± 30 |

High oxalate foods included chocolate, nuts, peanut butter

Oxalate content of food measured by Ion Chromatography couple with Mass Spectrometry after HCl extraction

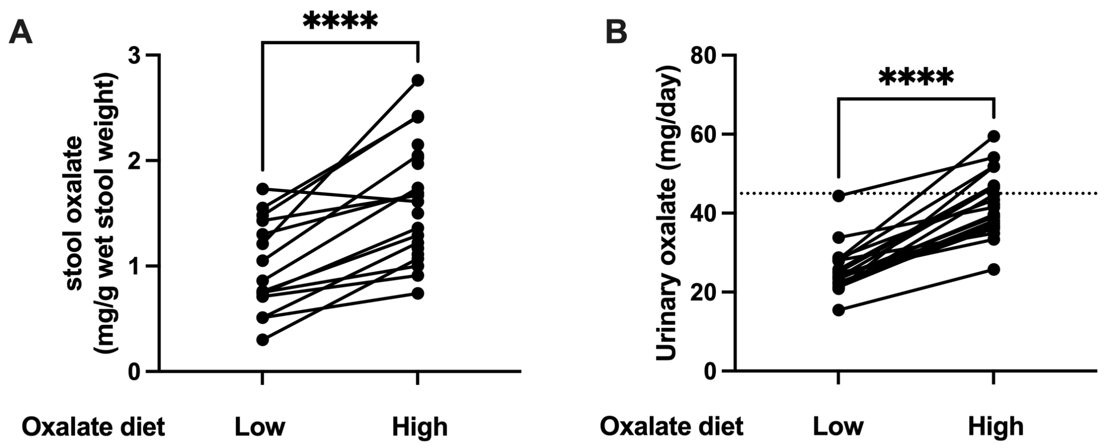


# *O. formigenes* - Inducing colonization study



**Results**

Effect of dietary oxalate on stool and urinary oxalate before colonization with *O. formigenes*




| | | |
|---|---|---|
| Response to the high oxalate diet | | |
| Urinary oxalate increase on high-oxalate diet (mg/day) | 17 ± 7 | [7 - 31] |
| Dietary oxalate absorption (% of dietary content) | 8 ± 3 | [3 - 15] |
| Stool oxalate increase on the high oxalate diet (mg/g wet stool weight) | 0.6 ± 0.4 | [-0.1 – 1.5] |

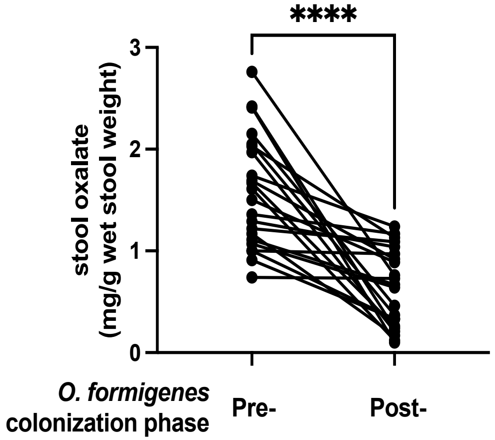
*NCT03752684, submitted*

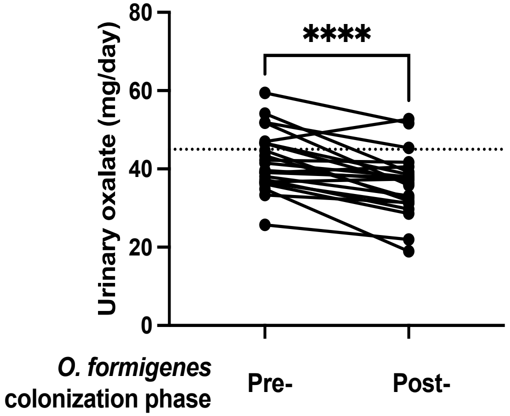
# *O. formigenes* - Inducing colonization study

## Results

Significant reduction in stool and urine oxalate post-colonization (54% and 14%, respectively, $p<0.001$)
No association of response with any parameter assessed

42±8 mg/d to 37±9 mg/d




| | | |
|---|---|---|
| Response to O. formigenes colonization | | |
| Urinary oxalate decrease post colonization (mg/day) | 6 ± 6 | [-6 - 16] |
| Urinary oxalate relative decrease post colonization (%) | 14 ± 13 | [-12 - +46] |
| Stool oxalate decrease post colonization (mg/g wet stool weight) | 1.0 ± 0.7 | [0.0 - 2.3] |

*NCT03752684, submitted*

# Conclusions



Colonization and re-colonization can be achieved, durably in some (up to 3-year follow-up study)

Humans are readily colonized with *O.formigenes* live cultures

*O.formigenes* colonization is sensitive to commonly prescribed antibiotics

*O.formigenes* colonization significantly reduces 24-hr urinary oxalate excretion on low calcium diets

# Outstanding Questions



What factors influence variability in response following colonization?

*Oxalobacter formigenes* strain differences?

Role of abundance and diversity of other potential oxalate-degrading bacteria, on impact of O. formigenes (microbiome molecular analyses)

What is the impact of colonization on urinary oxalate excretion in individuals with
- Idiopathic CaOx kidney stone disease? (study enrolling)
- Enteric hyperoxaluria?

# Summary

- Central role of *Oxalobacter formigenes* in dietary oxalate degradation
- Inducing stable colonization in humans is possible with a single ingestion of live *O. formigenes* cells
- Colonization with *O. formigenes* leads to reduced stool and urinary oxalate excretion in healthy volunteers on diets moderately rich in oxalate, low in calcium

# Future work

- Assessing the effect of colonization with *O. formigenes* in patient population (CaOx kidney stones, enteric hyperoxaluria)
- Understanding causes of variability in response to *O. formigenes*
- Role and impact of other gut microbiota on gut oxalate degradation and *O. formigenes*

# Acknowledgements

**UAB Kidney Stones & Urology Research Lab**

D Assimos, MD
M Bui
J Crivelli, MD
S Fargue, MD PhD
T Keenum
J Knight, PhD
X Li, PhD
D Wiley
K Wood, MD
S Zhou

UAB CCTS Clinical Research Unit & Bionutrition (*UL1TR003096)*

O'Brien center for AKI at UAB *(P30DK079337)*

UAB Nutrition and Obesity Research Center *(P30DK056336)*

A Miller, PhD at Cleveland Clinic Lerner Resarch Institute

U.S. Department of Health and Human Services

NIH National Institute of Diabetes and Digestive and Kidney Diseases

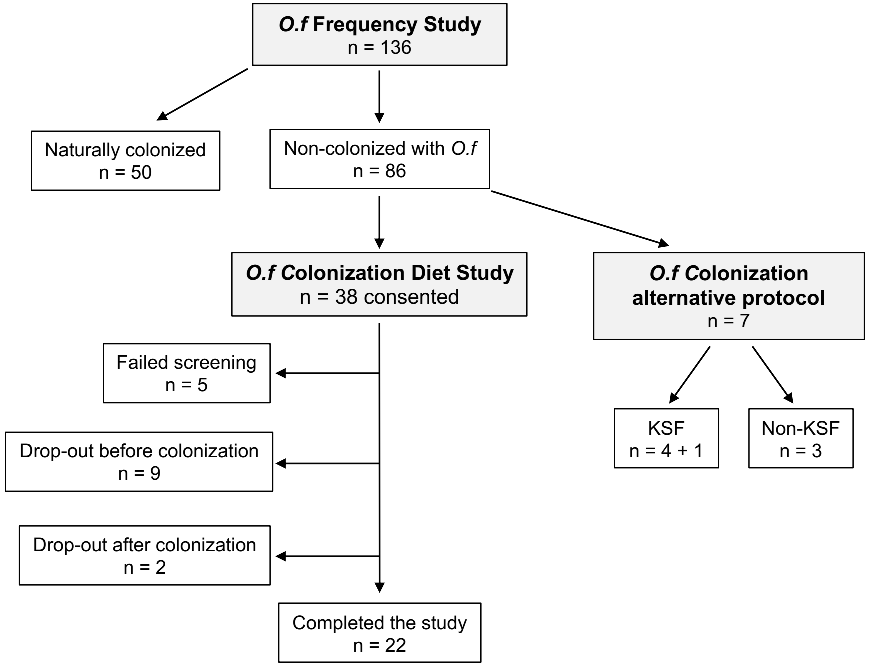
Oxalosis & Hyperoxaluria Foundation

Urology Care FOUNDATION
The Official Foundation of the American Urological Association

RARE KIDNEY STONE CONSORTIUM

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

# Thank you!




## *O.f* colonization in KSF

5 / 5 KSF successfully colonized with 1 dose of *O.f*

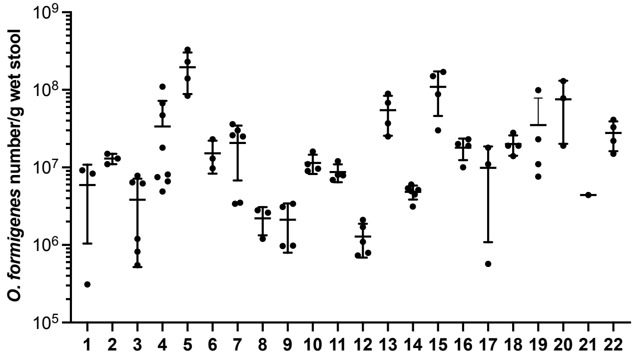
## *O.f* colonization study in healthy non KSF

22 / 22 successfully colonized with 1 dose of *O.f*

9 / 22 still colonized at 1 year post-dosing (up to 3 years follow-up)

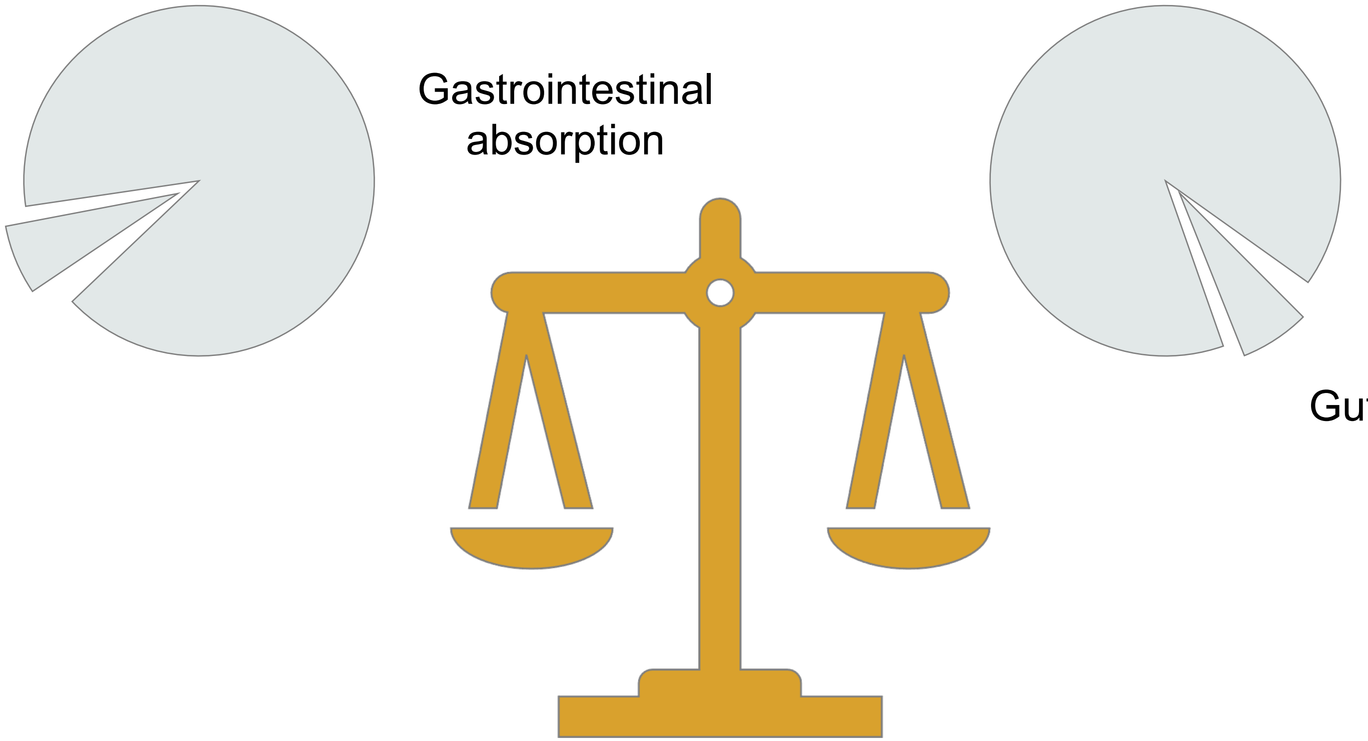
9 / 11 who lost *O.f* received antibiotics

6 redosed: 5/6 successfully recolonized




*O.F enumeration in stool (qPCR)*

# Oxalate (im)balance – Increased GI Absorption



# Oxalate – Increased Intake



**Increased intake** of dietary oxalate / precursors

BMC Nephrology

BMC Nephrol. 2023; 24: 207. PMCID: PMC10347717
Published online 2023 Jul 13. doi: 10.1186/s12882-023-03236-9 PMID: 37443012

Purslane-induced oxalate nephropathy: case report and literature review

Xiangtuo Wang, Xiaoyan Zhang, Liyuan Wang, Ruiying Zhang, Yingxuan Zhang, and Lei Cao

Author information Article notes Copyright and License information PMC Disclaimer

INDIAN JOURNAL OF NEPHROLOGY

Indian J Nephrol. 2016 Nov-Dec; 26(6): 446–448. PMCID: PMC5131384
doi: 10.4103/0971-4065.175978 PMID: 27942177

Acute kidney injury associated with ingestion of star fruit: Acute oxalate nephropathy

A. K. Barman, R. Goel, M. Sharma, and P. J. Mahanta

Arch Pathol (Chic). 1946 Jun:41:631-8.

Ethylene glycol poisoning; with suggestions for its treatment as oxalate poisoning

G MILLES

PMID: 20993494

Case Reports Clin Nephrol. 2017 Dec;88(12):354-358. doi: 10.5414/CN109118.

Oxalate nephropathy following vitamin C intake within intensive care unit

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PMID: 29092737 DOI: 10.5414/CN109118

Abstract

**Objective:** To report a case of acute oxalate nephropathy related to vitamin C intake within the intensive care unit (ICU).

**Design:** Case report.

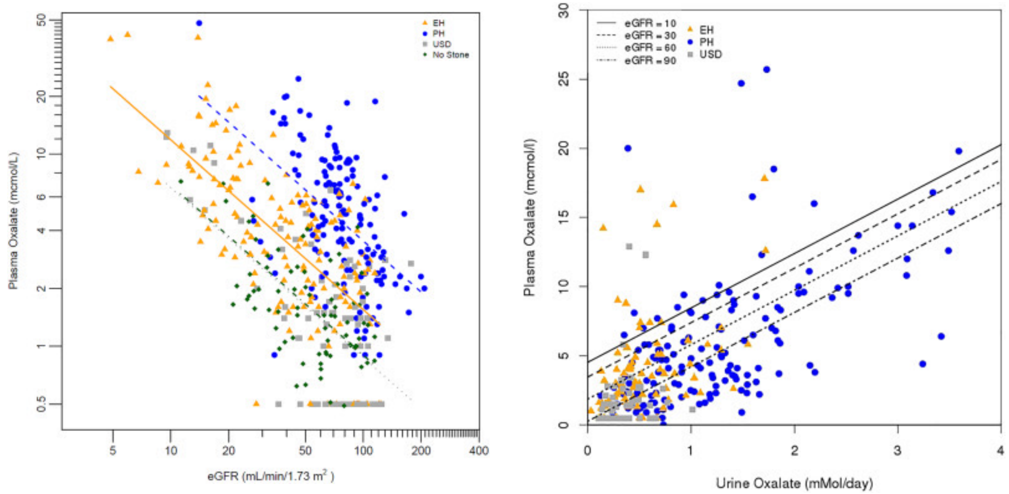
# Oxalate – Increased GI Absorption



**Enteric hyperoxaluria**

GI disorders (gastric bypass, chronic pancreatitis, coeliac disease, IBD) causing fat malabsorption and/or inflammation, etc.)

Maybe greater GI absorption of oxalate in **KSF** *(how and where?)*




*Perinpam Clin Biochem 2017*

*Langman, cJASN 2023, Witting cJASN 2021, Perinpam Clin Biochem 2017*

# Oxalate – Increased GI Absorption



**Enteric hyperoxaluria**

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| Study | participants enrolled (n) | | | BMI (kg/m2) | Controlled diet (mg Oxalate/ Calcium) | Oxalate Load | Oxalate absorption (%) | | | Hyperabsorber | % Oxalate absorbed in 6h | Comment/limitations |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HV | KSF | other | | | | HV | KSF | other | | | |
| **Siener, 2024** | 27 | 18 Crohn | 9 Crohn NSF | 18-26 | Yes, no detail | 50 mg 13C2-Ox | 7.2 ± 4.2 | 17.4 ± 8.1 | 9.4 ± 10.3 | 100% of Crohn | 50-80% | 83-96% absorbed within 12-hr |
| **Knight, 2011** | 0 | 38 | | <35 | no | 13C2-Ox 90 mg + 5 g Sucralose | - | 8.7 ± 5.9 | | 5/38 [a] | 67% | soluble oxalate, no Controlled Diet, no healthy group, no BMI adjustment |
| **Sikora, 2008** | 35 | 60 | | - | 60/800 | 25/50 mg 13C2-Ox | 10.4 [1.9-26.2] | 15.3 [1.7-37.7] | | 23/60 | - | soluble oxalate, pediatric, only 36 CaOx KSF characterized, no BMI adjustment |
| **Thomas, 2007** | 8 | 0 | | 18-30 | 60/700 & 300/1000 | 50 mg 13C2-Ox | 9.2 ± 2.7 & 12.5 ± 4.6 | - | | - | - | no KSF, diet composition impacting load |
| **Voss, 2006** | 120 | 120 | | 18-32 | 63/800 | 50 mg 13C2-Ox | 8.0 ± 4.4 | 10.2 ± 5.2 | | 12 HV & 19 KSF [a] | 80-85% | soluble oxalate, no BMI adjustment, no mechanistic approach |
| **von Unruh, 2003** | 120 | 0 | | 18-32 | 63/800 | 50 mg 13C2-Ox | 7.9 ± 4.0 | - | | yes | 85% | soluble oxalate, no BMI adjustment |
| **Chai, 2004** | 9 | 2 | | 25 ± 5 | no (food list) | 20 mg 13C2-ox + 100 mg food oxalate | 6.7 ± 0.4 / 7.0 ± 0.4 | - | | - | 83% | small numbers, no KSF data, no BMI adjustment |
| **Hesse, 1999** | 50 | 70 | | - | Yes, no detail | 50 mg 13C2-Ox | 6.7 ± 3.9 | 9.2 ± 5.1 | | 20% HV & 34% of KSF [b] | - | soluble oxalate, no BMI adjustment |

Gastrointestinal absorption in KSF in the literature. Results mean ± SD if provided. (** p<0.001, * p<0.05).

# Oxalate (im)balance – Increased GI Absorption



**TABLE 3. Likelihood of Being an Incident Symptomatic Stone Former by Each Nutrient Intake Among 411 Stone Formers and 384 Controls[a]**

| | Unadjusted | | Adjusted for age, sex, race, fluid and energy intake | | Adjusted for age, sex, race, fluid and energy intake, and BMI | |
|---|---|---|---|---|---|---|
| Nutrient intake per SD | OR (95% CI) | *P* value | OR (95% CI) | *P* value | OR (95% CI) | *P* value |
| Calcium (mg/d)[b] | | | | | | |
| <1200 | 0.46 (0.33-0.66) | **<.001** | 0.44 (0.31-0.65) | **<.001** | 0.43 (0.29-0.63) | **<.001** |
| ≥1200 | 1.11 (0.88-1.40) | .40 | 1.11 (0.87-1.41) | .41 | 1.12 (0.88-1.44) | .36 |
| Fluid (mL/d)[b,c] | | | | | | |
| <3400 | 0.74 (0.58-0.95) | **.02** | 0.70 (0.54-0.91) | **.007** | 0.65 (0.50-0.85) | **.002** |
| ≥3400 | 1.42 (1.05-1.93) | **.03** | 1.43 (1.04-1.95) | **.03** | 1.35 (0.98-1.86) | .063 |
| Potassium (mg/d) | 0.74 (0.64-0.86) | **<.001** | 0.66 (0.55-0.79) | **<.001** | 0.67 (0.56-0.80) | **<.001** |
| Sodium (mg/d) | 0.95 (0.83-1.09) | .48 | 1.00 (0.86-1.16) | .98 | 0.92 (0.79-1.08) | .31 |
| Animal protein (g/d) | 0.97 (0.84-1.11) | .62 | 0.97 (0.84-1.12) | .67 | 0.91 (0.78-1.06) | .22 |
| Vegetable protein (g/d) | 0.91 (0.79-1.05) | .20 | 0.92 (0.79-1.06) | .24 | 0.94 (0.81-1.10) | .43 |
| Caffeine (mg/d) | 0.90 (0.79-1.04) | .15 | 0.83 (0.70-0.98) | **.03** | 0.84 (0.71-0.99) | **.04** |
| Fiber (mg/d) | 0.98 (0.85-1.13) | .78 | 0.98 (0.84-1.14) | .81 | 1.02 (0.87-1.19) | .85 |
| Phytate (mg/d) | 0.76 (0.65-0.88) | **<.001** | 0.74 (0.64-0.87) | **<.001** | 0.76 (0.65-0.89) | **.001** |
| Oxalate (mg/d) | 0.98 (0.85-1.13) | .76 | 0.97 (0.84-1.12) | .64 | 1.01 (0.87-1.17) | .94 |

[a]BMI, body mass index; OR, odds ratio.
[b]Nutrient intake modeled using a piecewise linear regression term, with cutoff determined by Akaike Information Criterion metric.
[c]Not adjusted for fluid intake.
Odds ratios and *P* values in boldface print denote statistical significance at the .05 α level.

*Taylor et al. cJASN 2008*