The Impact of Quaternary Ammonium Compounds on Gut Microbiome And Gut-liver Interactions

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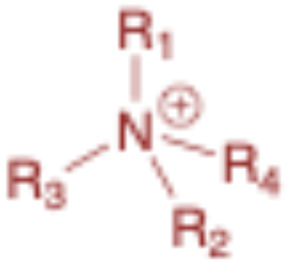
What are Quaternary Ammonium Compounds (QACs)?

Cleaning products

Medical products

Food processing



(QACs)

(BACs)

(ATMAC)

(CTPC)

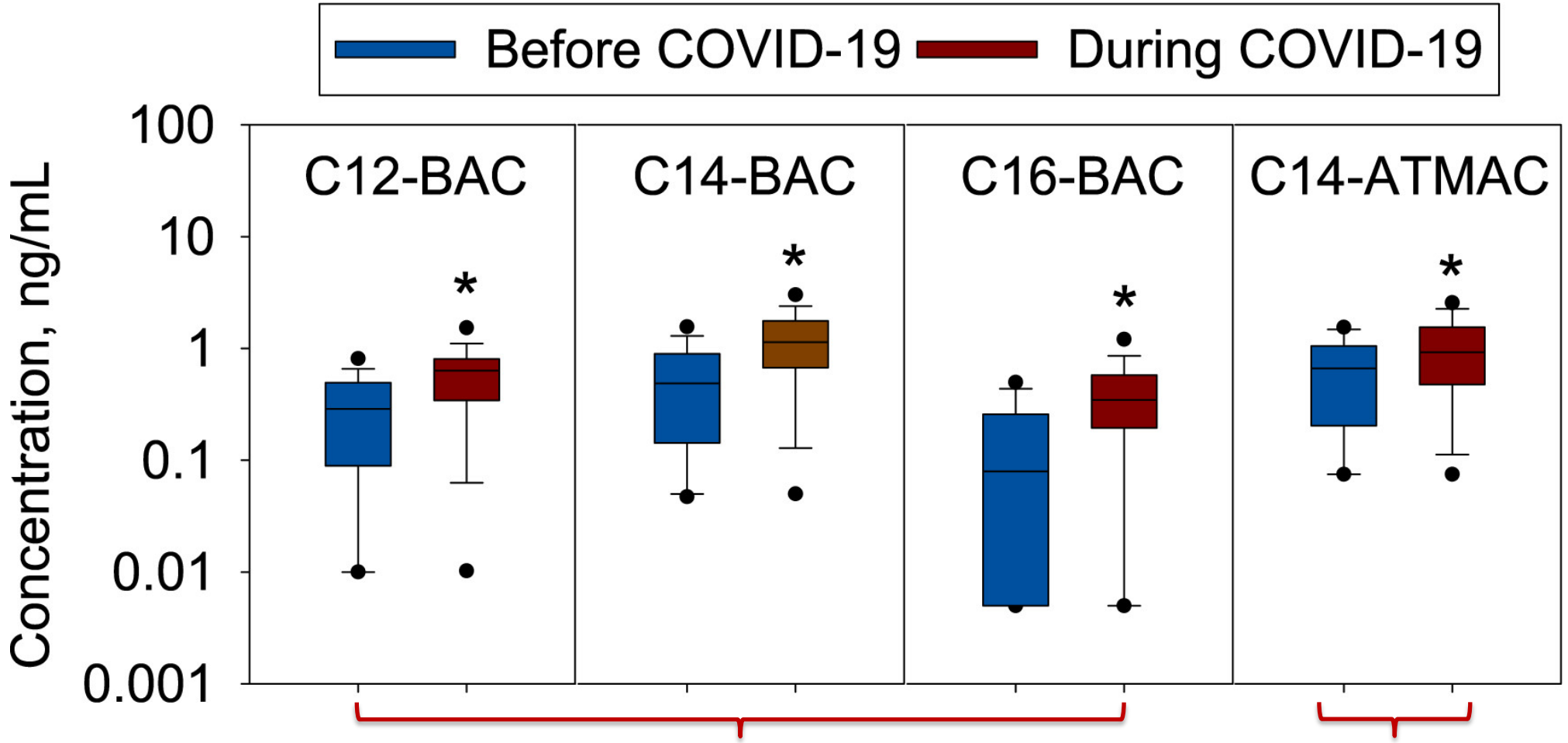
(DDAC)

Disinfectants, pesticides, preservatives

Arnold *et al*. Quaternary Ammonium Compounds: A Chemical Class of Emerging Concern. *Environ. Sci. Technol.* **57**, 7645–7665 (2023).

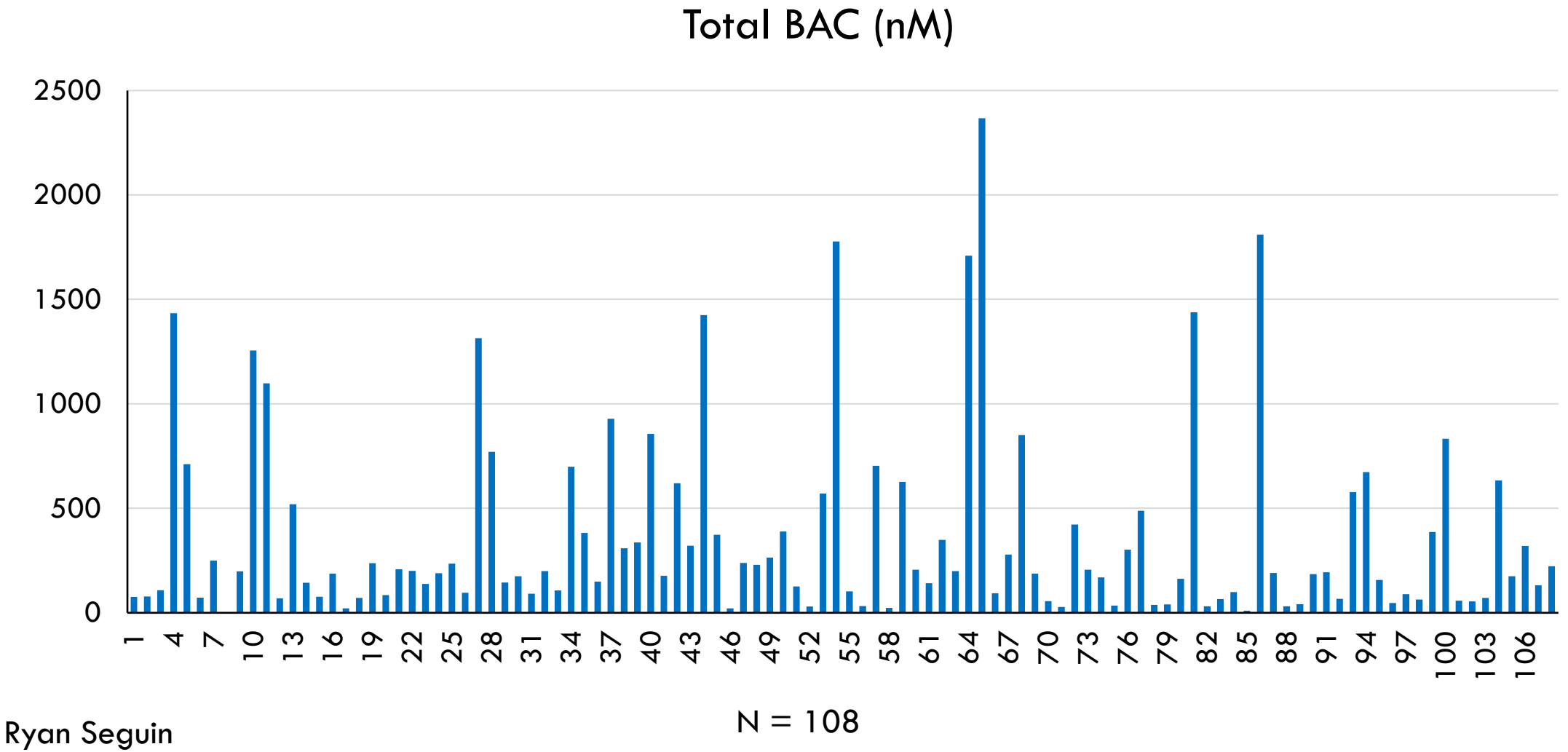
Are QACs found in humans?

Median QAC Levels in Human Blood Have Increased Relative to Pre-COVID



Zheng et al. *Environ. Sci. Technol.* **2021**, 55:14689-14698

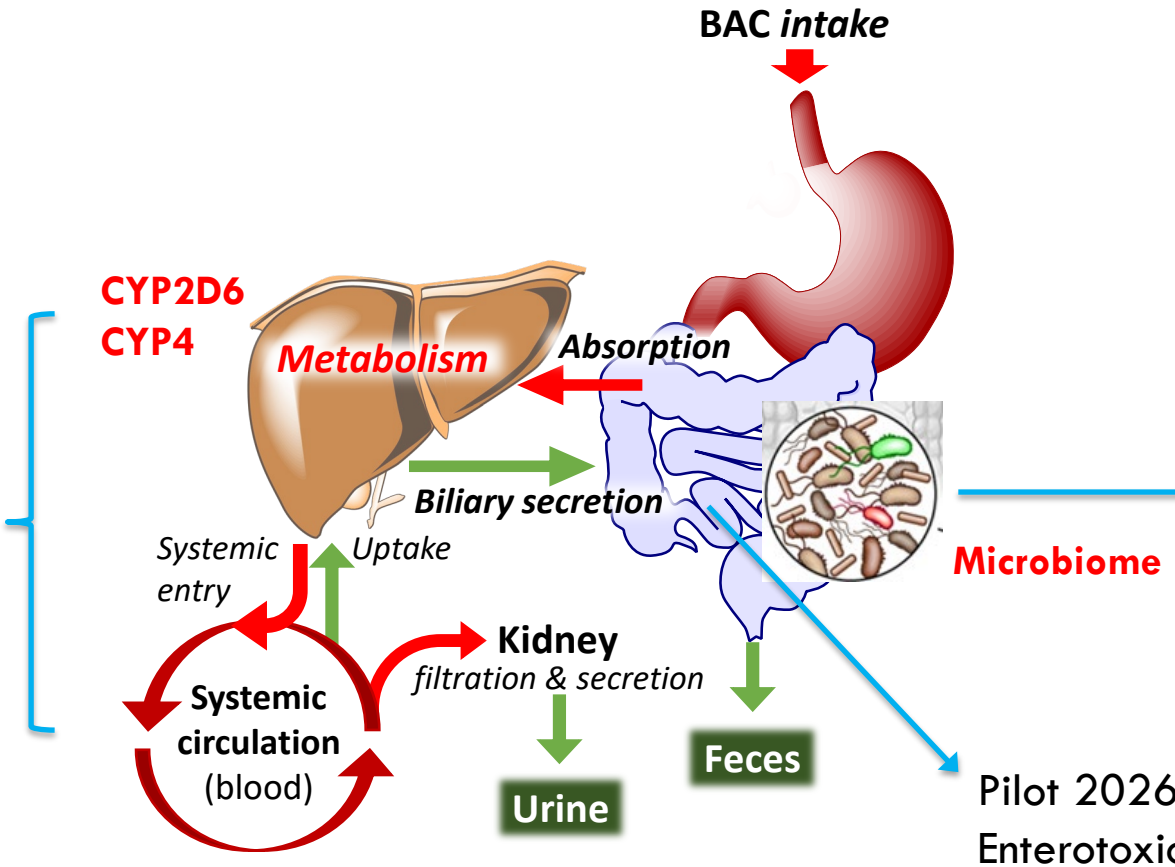
Parent BACs Reach Up to µM in Human Feces



Ryan Seguin

Overview of BAC Metabolism and Disposition

R01ES031927 (2021-2026): Metabolism, Transport, and Kidney Toxicity



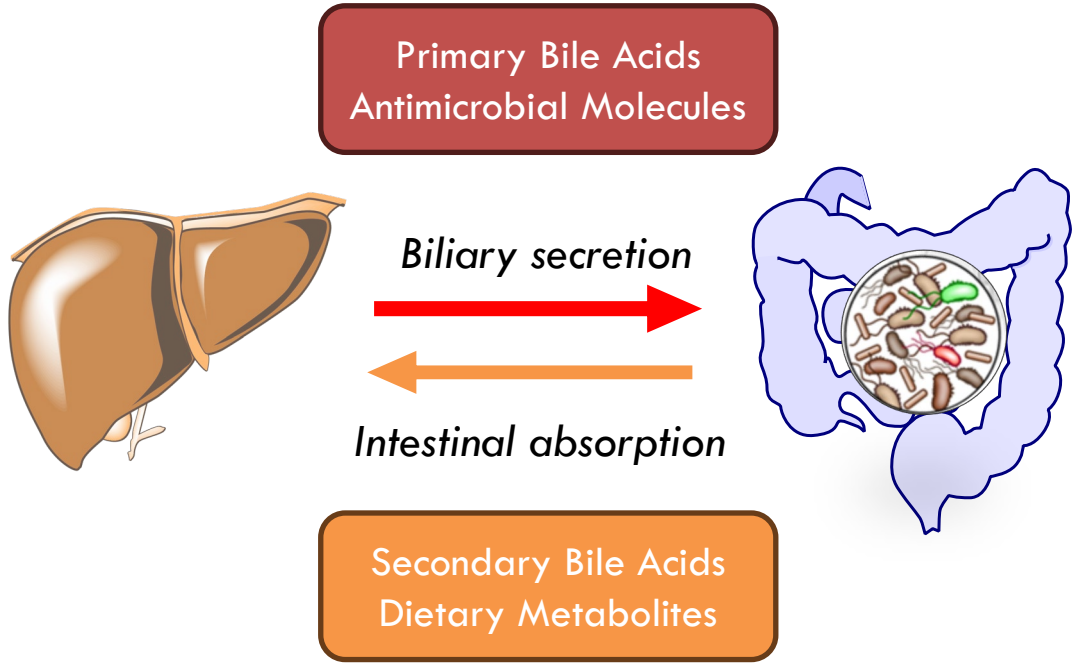
R01ES035021 (2024-2029): Impact of benzalkonium chloride on gut microbiome and gut-liver interactions

Pilot 2026 (Xu and Kelly): Enterotoxicity of benzalkonium chloride

Figure by Ryan Seguin

Seguin et al. (**2019**) *Chem. Res. Toxicol.*, 32(12), 2466–2478.
Seguin, R., and Xu, L. (**2022**) *ASPET 2022 Annual Meeting.*
Lopez *et al. Toxicol. Sci.* (**2024**) 202, 265–277.
Brzoska, et al. *ES&T* (**2026**), In Press.

Bidirectional Interactions Between Gut and Liver



Hypothesis: BACs reduce gut microbiome diversity and alter the metabolism of xenobiotics, bile acids, sterols, and lipids in the liver by modulating the activities of nuclear receptors.

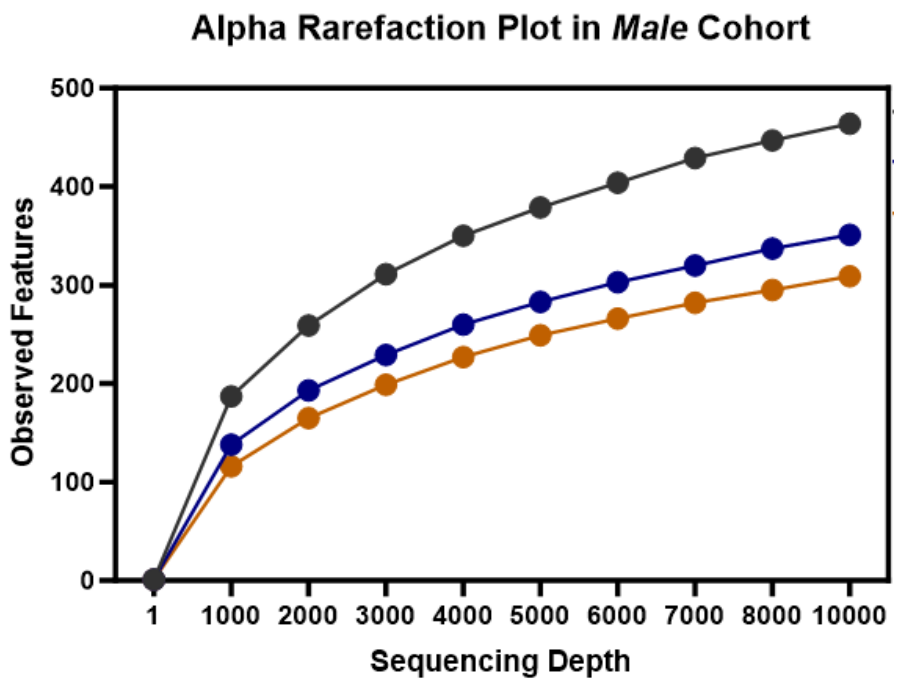
Tripathi, A. *et al. Nat Rev Gastroentero* **15**, 397–411 (2018).

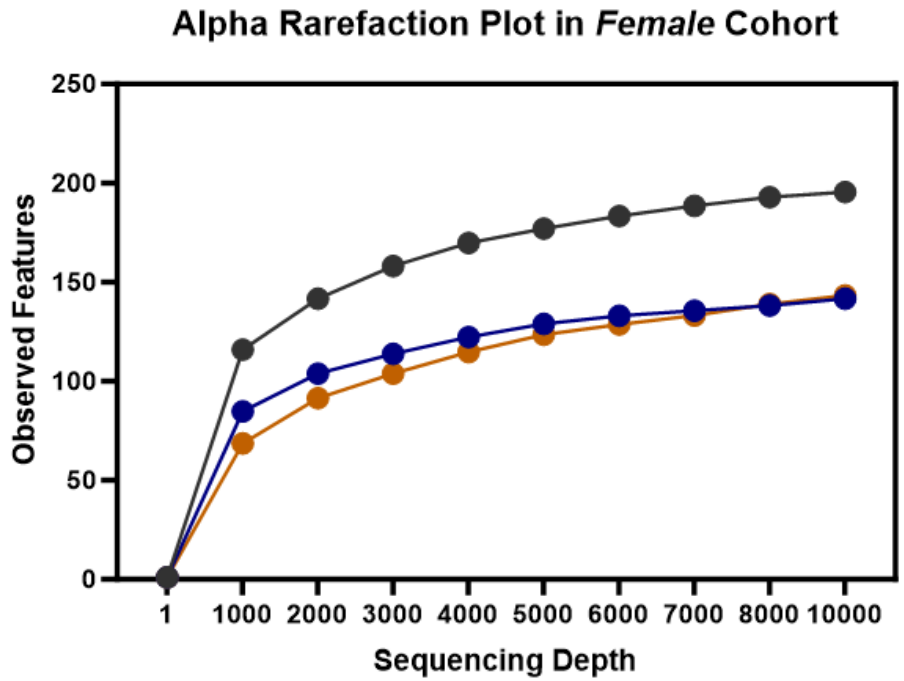
Specific Aims

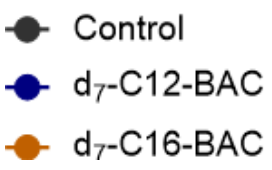
- Aim 1. Characterize the impact of BAC exposure on gut microbiome diversity and function in conventional mice.
- Aim 2. Measure the effects of BAC exposure on bile acid, sterol, lipid, and xenobiotic metabolism in the liver of conventional and germ-free mice.
- Aim 3. Evaluate the relationship between BAC levels and gut microbiome diversity and function in humans.

Aim 1a. Oral BAC Exposure Decreased Alpha Diversity of Gut Microbiome – 7-Day Exposure at 120 µg/g/day

16S rRNA sequencing







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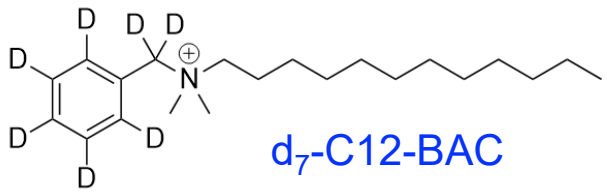
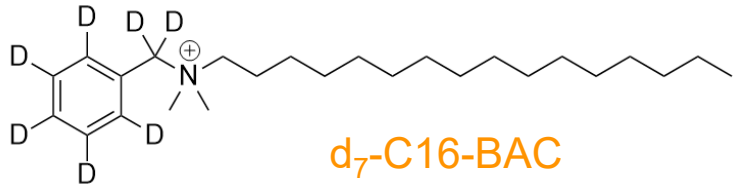


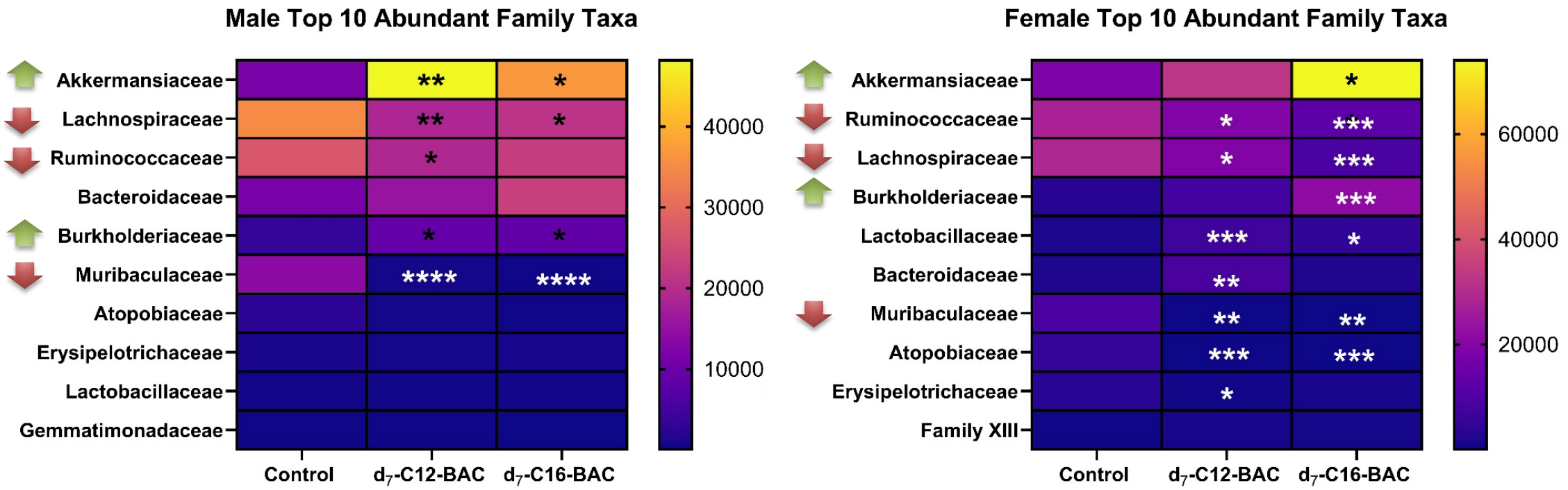
d_7-C12-BAC



d_7-C16-BAC

Lopez, V. A. *et al. Toxicol. Sci.* 202, 265–277 (**2024**).

Compositional Changes by BACs at the Family Level: 120 µg/g/day for 7 Days

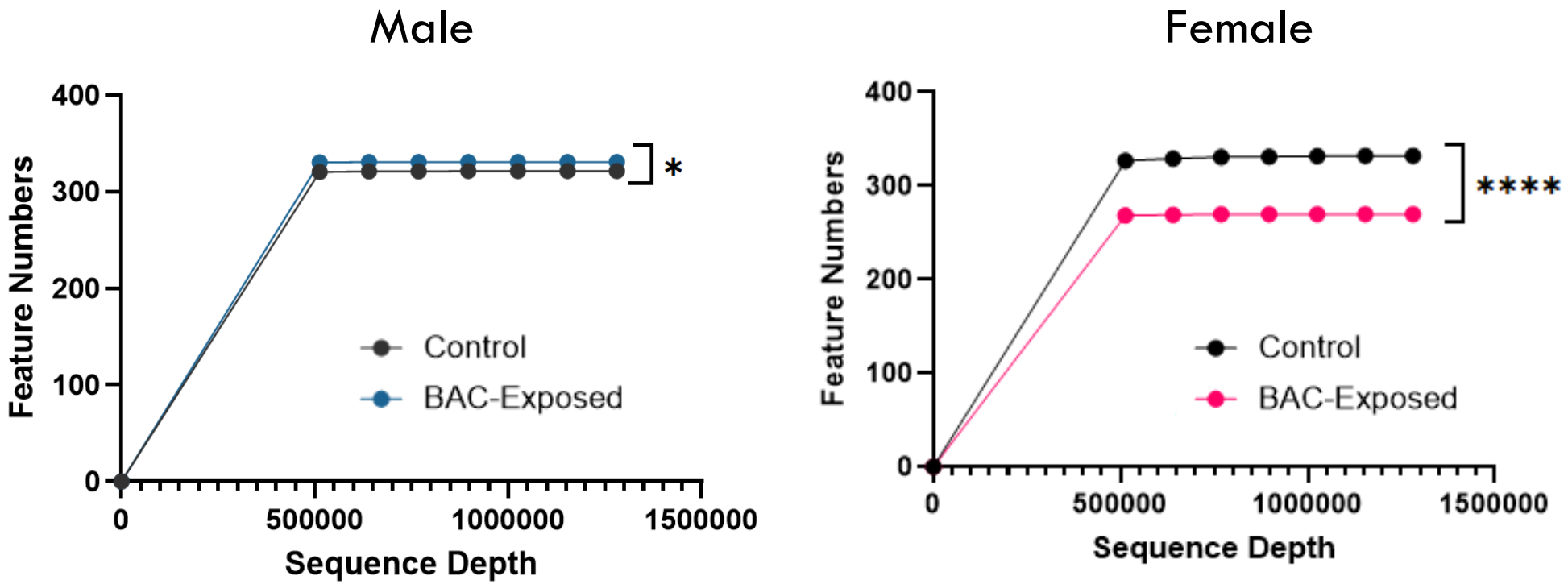


Ruminococcaceae and Lachnospiraceae are families of bacteria that can metabolize primary bile acids into secondary bile acids

Lopez, V. A. *et al. Toxicol. Sci.* 202, 265–277 (**2024**).

Aim 1b. Chronic Oral BAC Exposure Decreased Alpha Diversity of Gut Microbiome in Females – 31-Day Exposure at 1.2 µg/g/day

Metagenomics sequencing

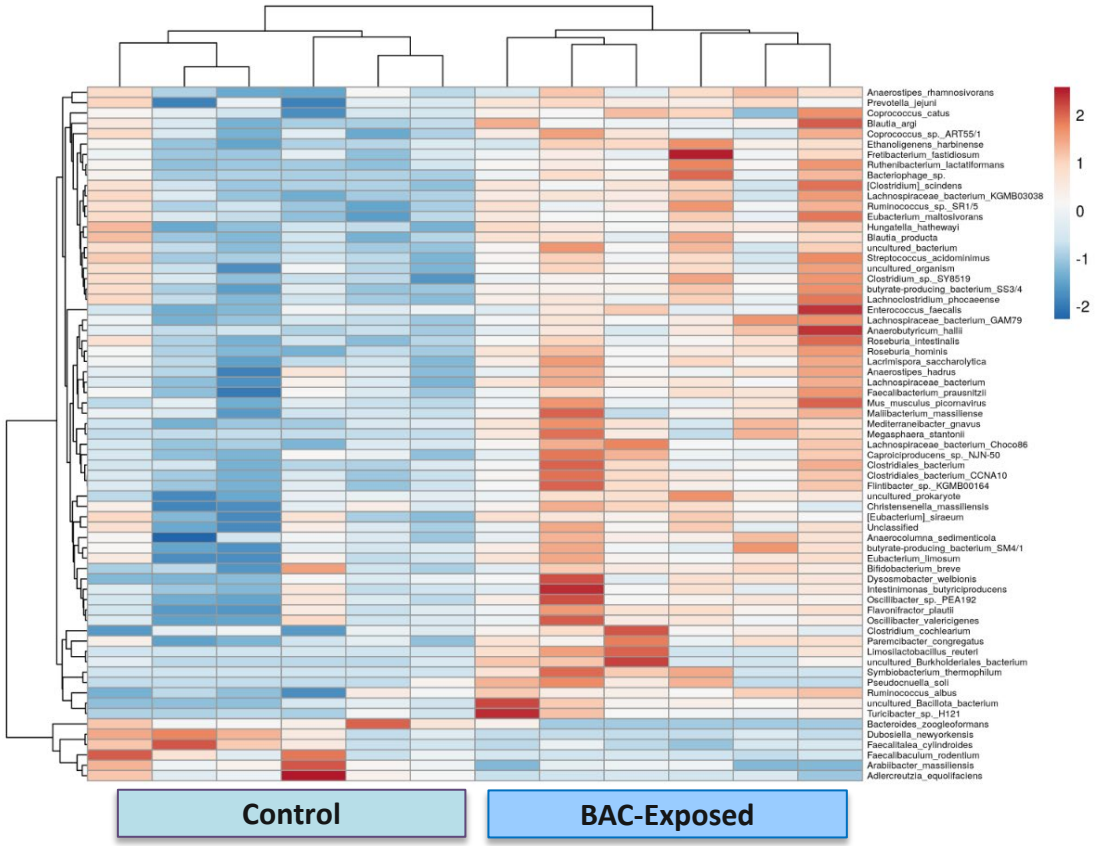


Lopez et al.
Unpublished (**2026**)

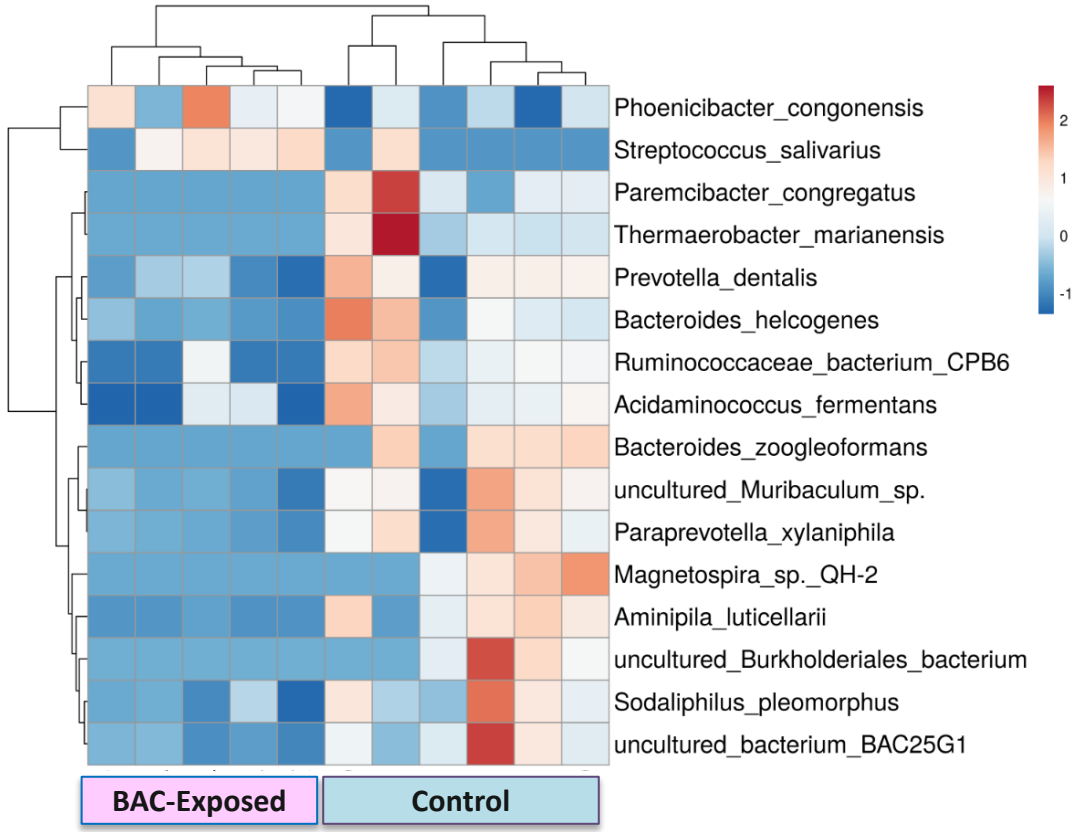
A mix of BACs was used: 40% C12, 50% C14-, and 10% C16-BAC: 1.2 µg/g/day in total.

Compositional Changes at the Species Level: Chronic Exposure at 1.2 µg/g/day

Male Cohort



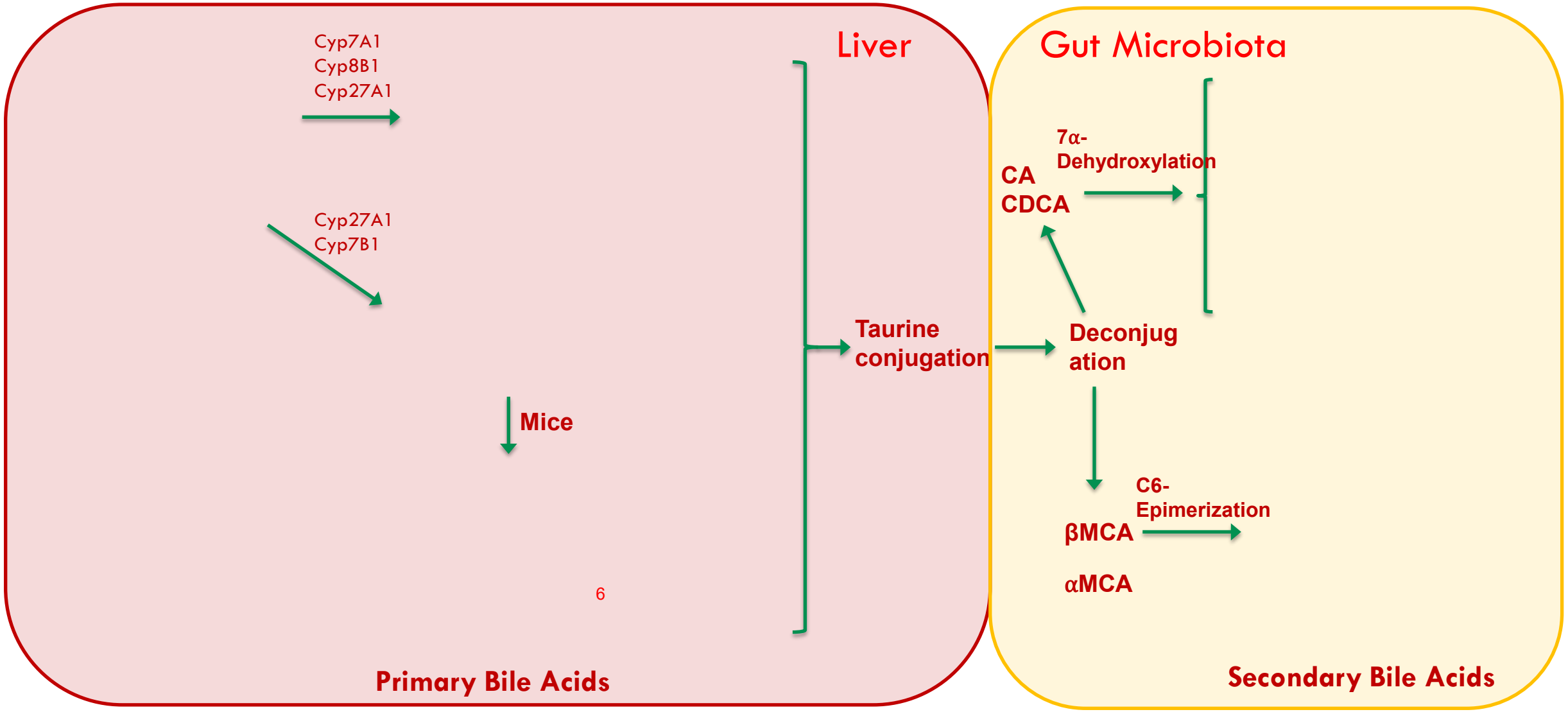
Female Cohort



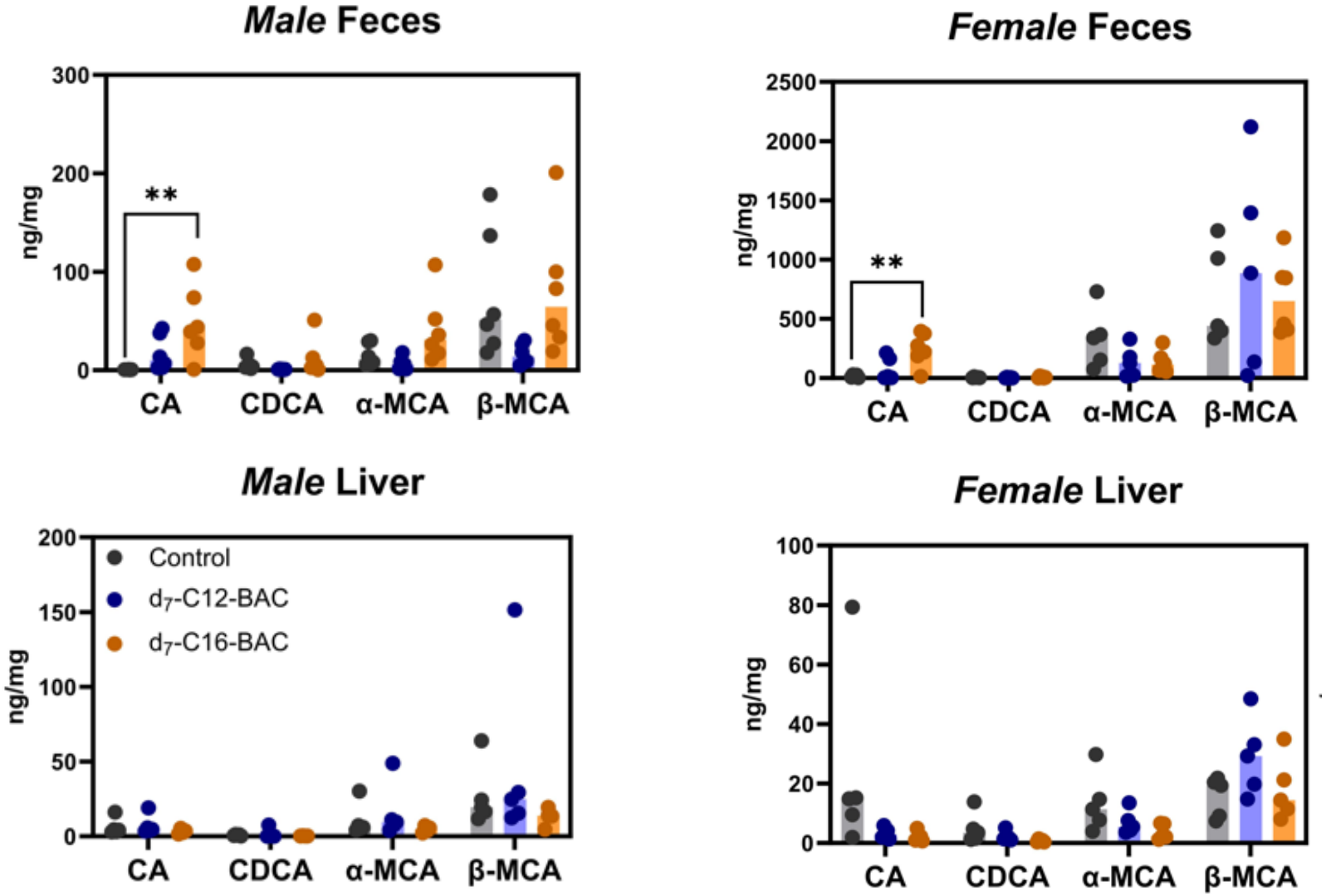
Lopez et al.
Unpublished (**2026**)

Changes in Bile Acid Metabolism

Bile Acid Metabolism in Mice

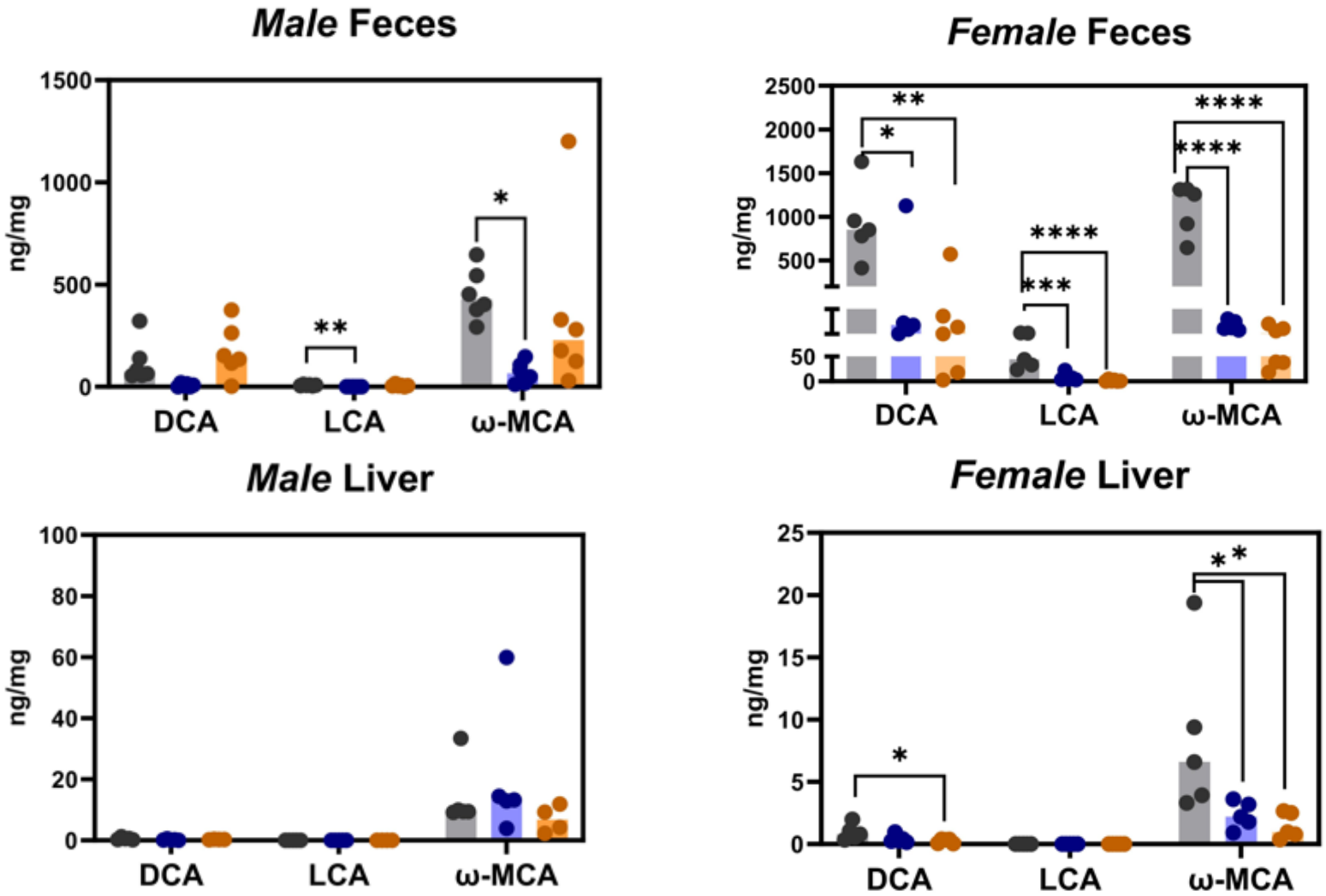


Small Increases in Primary Bile Acid Cholic Acid in Feces –120 µg/g/day Exposure



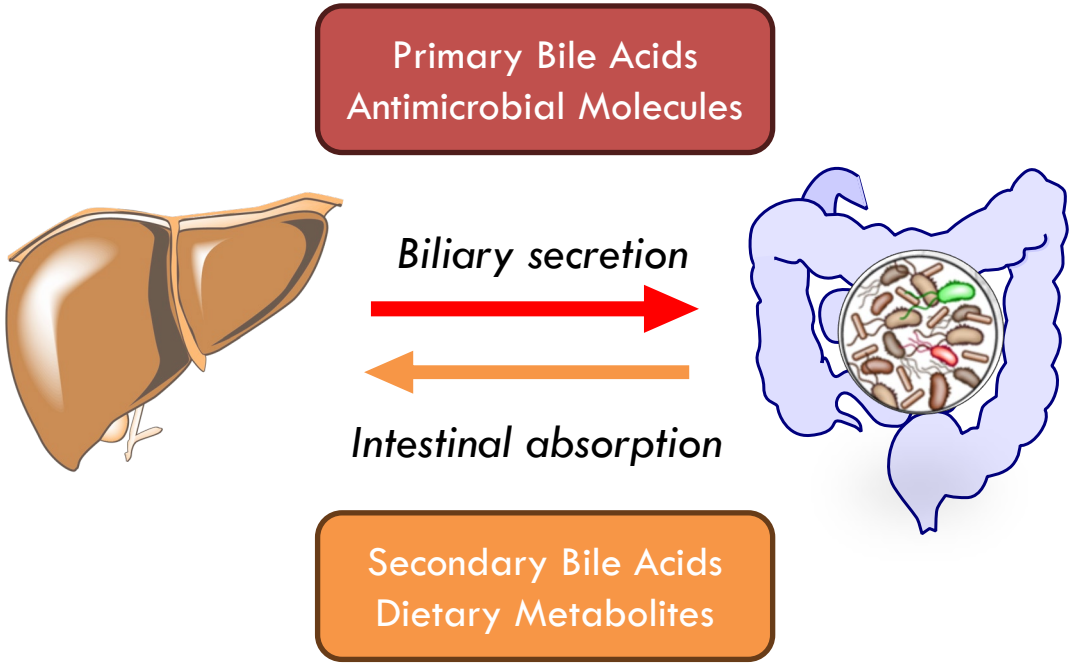
Lopez, V. A. *et al. Toxicol. Sci.* 202, 265–277 (**2024**).

Large Decreases in Secondary Bile Acids –120 µg/g/day Exposure

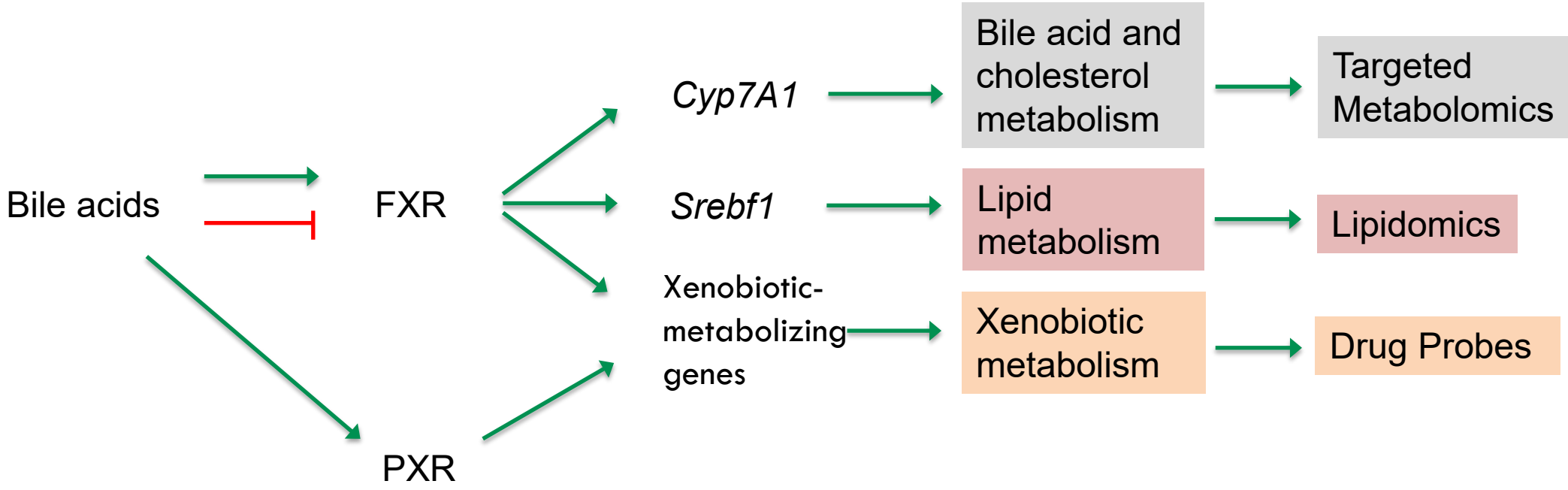


Lopez, V. A. *et al. Toxicol. Sci.* 202, 265–277 (**2024**).

How About Changes in the Liver? Aim 2

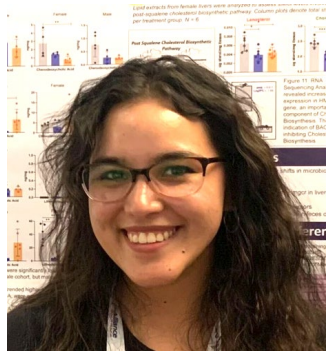


Bile Acid Action through Nuclear Receptors: Farnesoid X Receptor (FXR) and Pregnane X Receptor (PXR)



Pan, X.,. *Drug Metab Dispos* 43, 1002–1007 (2015).
Zhang, S., *Drug Metab Dispos* 43, 743–748 (2015).
Staudinger *Proc National Acad Sci* 98, 3369–3374 (2001).
Wang, Z.. *Mol. Med.* **27**, 144 (2021).

Cholesterol – 120 µg/g/day Exposure



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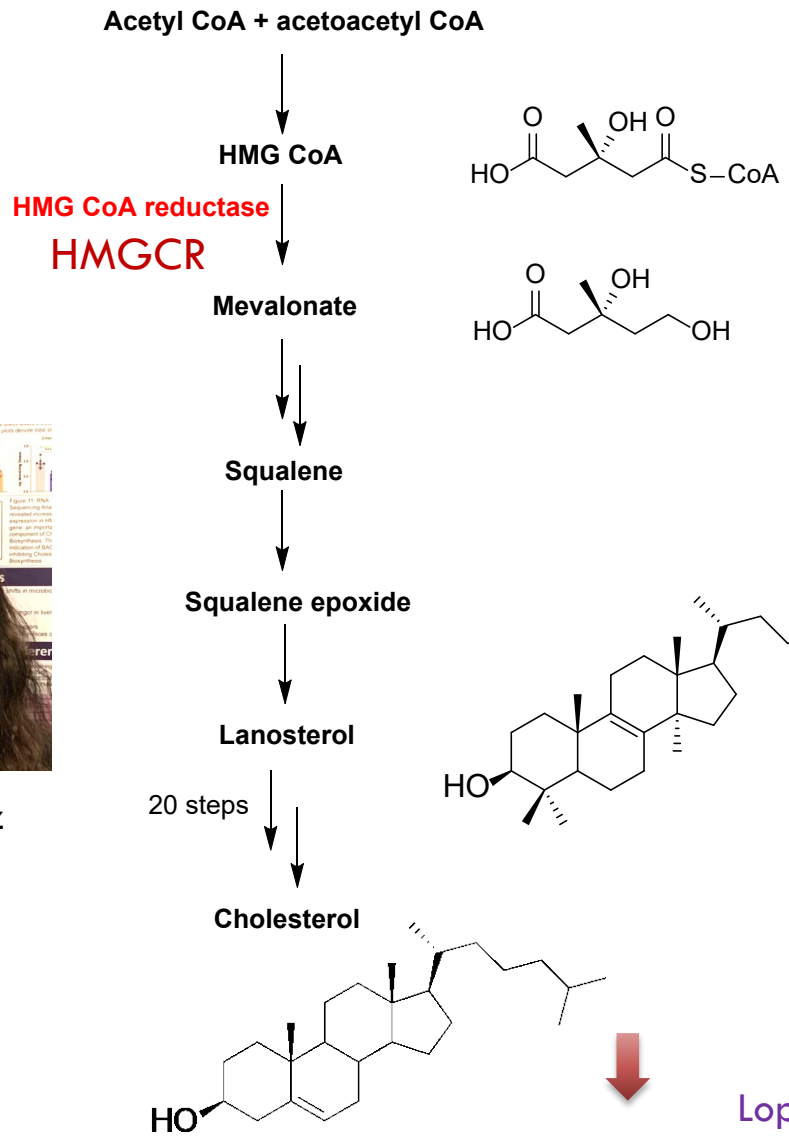
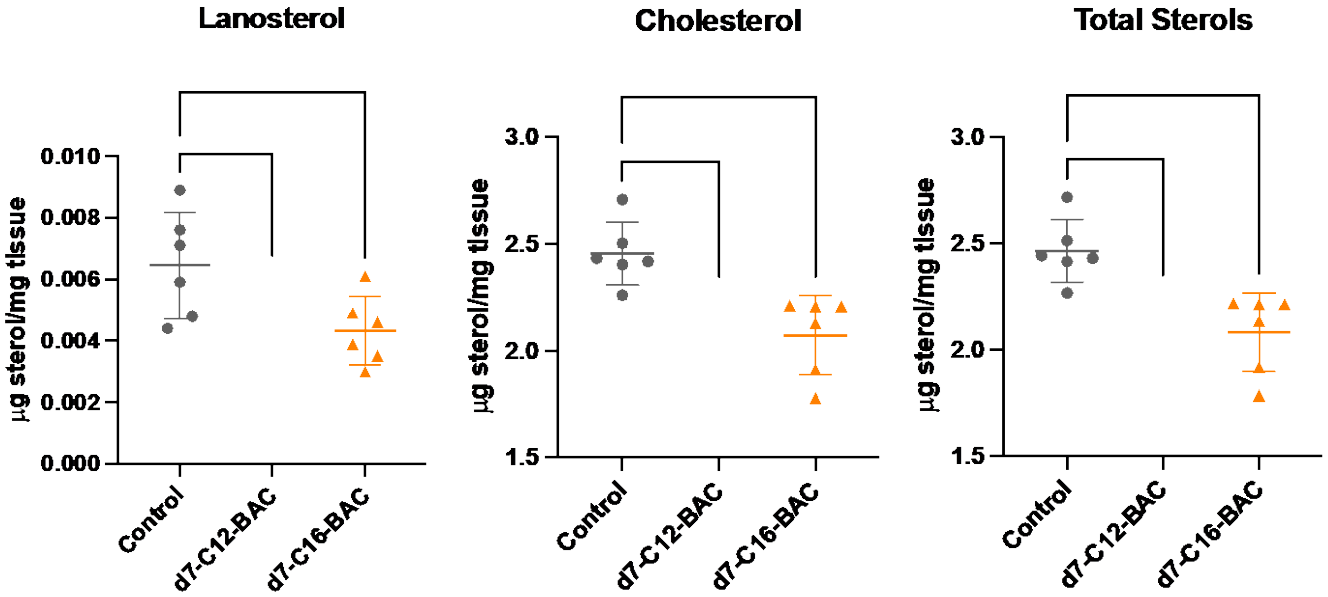
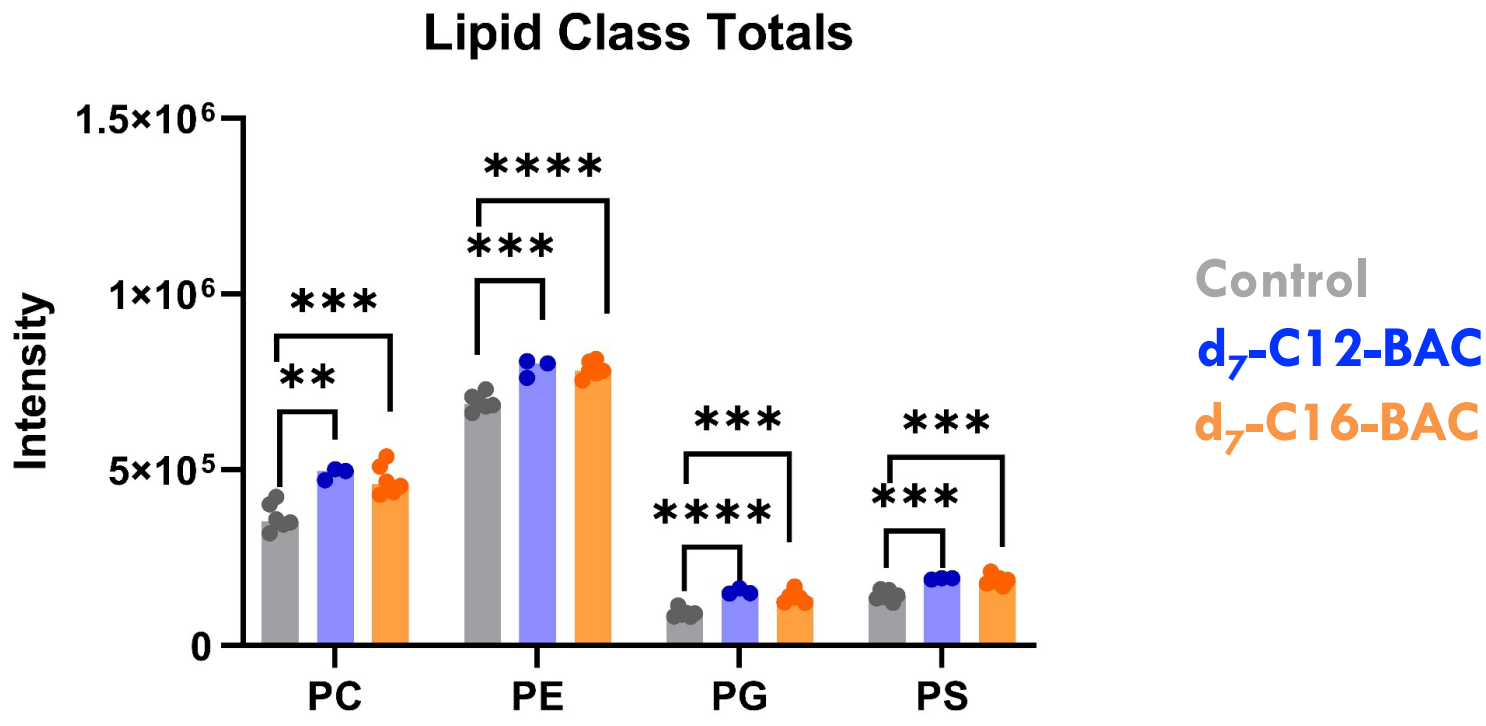




Suggesting Inhibition of cholesterol biosynthesis

Lopez et al. Unpublished (**2025**)

Lipidomics Revealed Upregulation of Major Phospholipid Classes – 120 µg/g/day Exposure

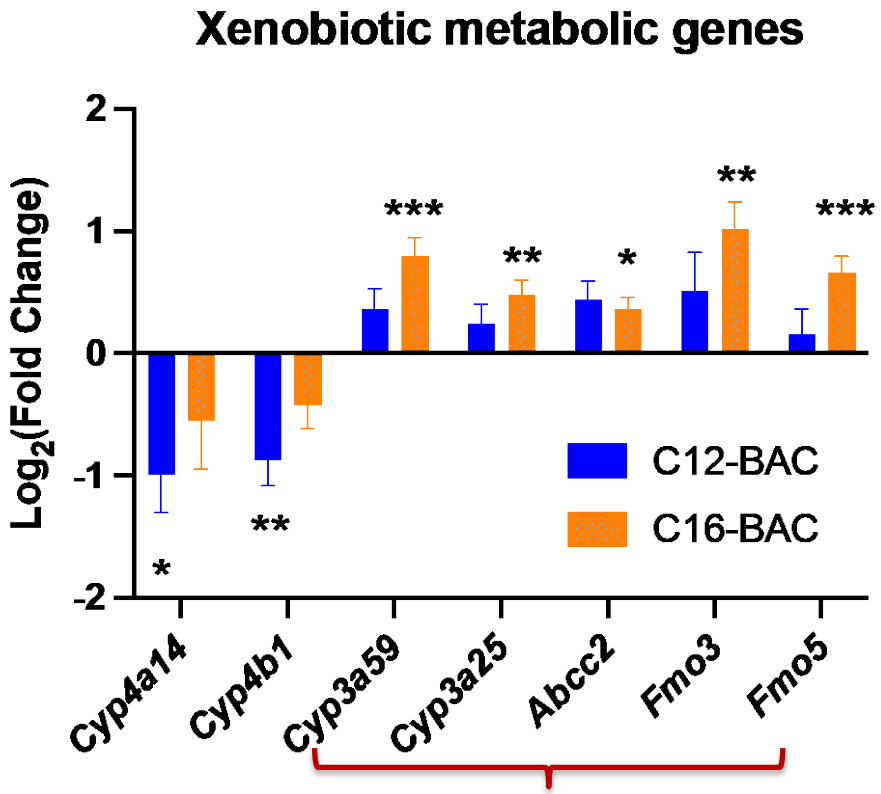


Phosphatidylcholine (PC)
Phosphatidylethanolamine (PE)
Phosphatidylglycerol (PG)
Phosphatidylserine (PS)

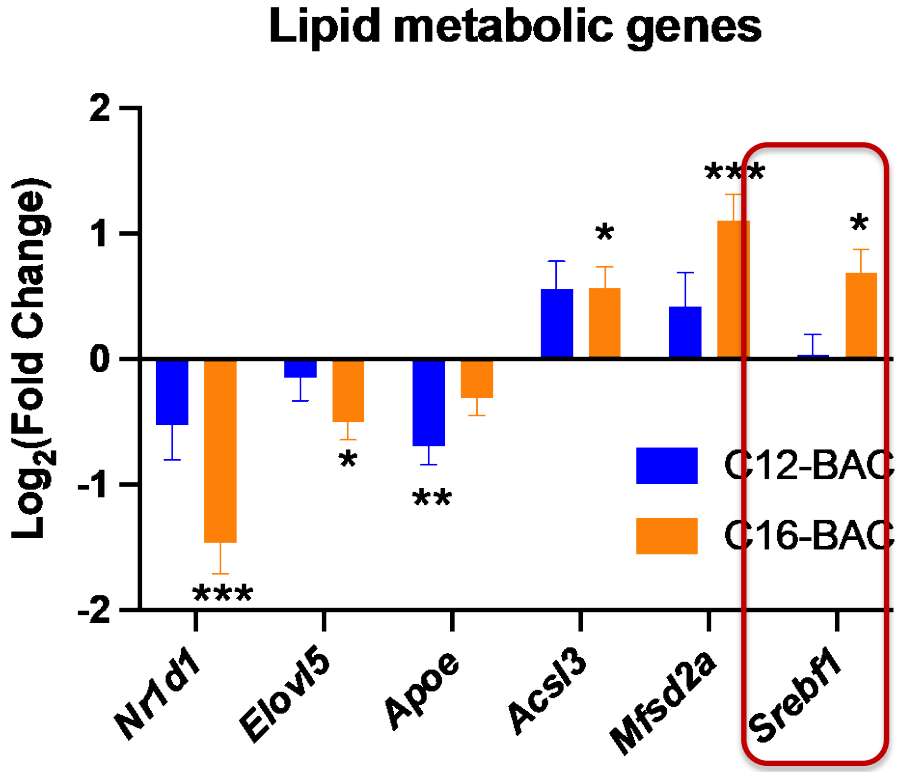
Lopez et al. Unpublished (**2025**)

RNA Seq Also Revealed Alteration in Xenobiotic and Lipid Metabolism – 120 µg/g/day Exposure

Lopez et al.
Unpublished



Possible activation of PXR and/or CAR



Master regulator of lipid synthesis

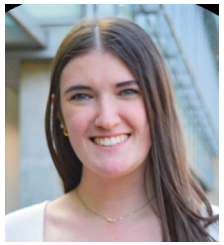
Cui, J. Y. & Klaassen, C. D. *Biochim Biophys Acta* 1859, 1198–1217 (2016).

Summary

- QACs are observed in human feces at up to µM concentrations.
- BACs, in general, reduce gut microbiome diversity.
- Alteration in gut microbiota composition led to reduced production of secondary bile acids.
- BAC-exposed livers display altered cholesterol, lipid, and xenobiotic metabolism

Acknowledgements

Scott Katz



Gabby Kunzman

Sydney Arnzen

- (Left) **Ryan Nguyen**, Linxi Zhu, Anvitaa Anandkumar, Corrina Cooper, Katya Lukyanova, Tianwei Shen
- Griffin Rangel, Rutan Zhang, **Ryan Seguin**, **Vanessa Lopez**, Noelle Reimers, Libin

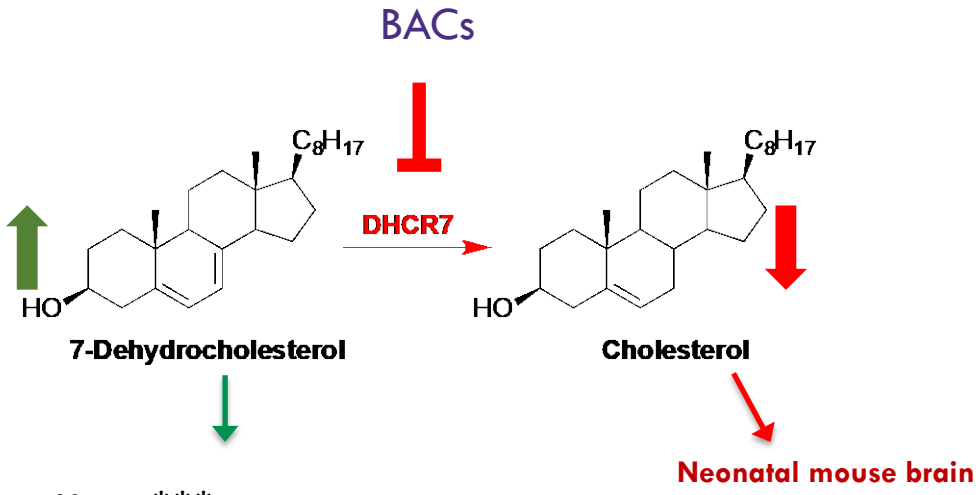
- School of Pharmacy Mass Spectrometry Center (Dale Whittington, Scott Edgar(in memorial))
- **Funding:** NIH (**R01ES035021**, **R01ES031927**, R01AI136979), **EDGE Pilot Grants** (2016, 2022, 2026), SOP Innovation Fund, UW Royalty Research Fund

NIH NIEHS

National Institute of Allergy and Infectious Diseases

QACs Display a Variety of Toxicities

Inhibit Cholesterol Biosynthesis



Herron et al. *Tox. Sci.* 2016

Herron et al. *Tox. Sci.* 2019

Cause Neural Tube Defects

Control QAC

Hrubec et al. *Birth Def. Res.* 2017

Josi Herron

Inhibits Mitochondrial Functions

QAC

Datta et al. *EHP*. 2017

Other effects:

Humans:

- Asthma
- Skin inflammation
- Eye inflammation

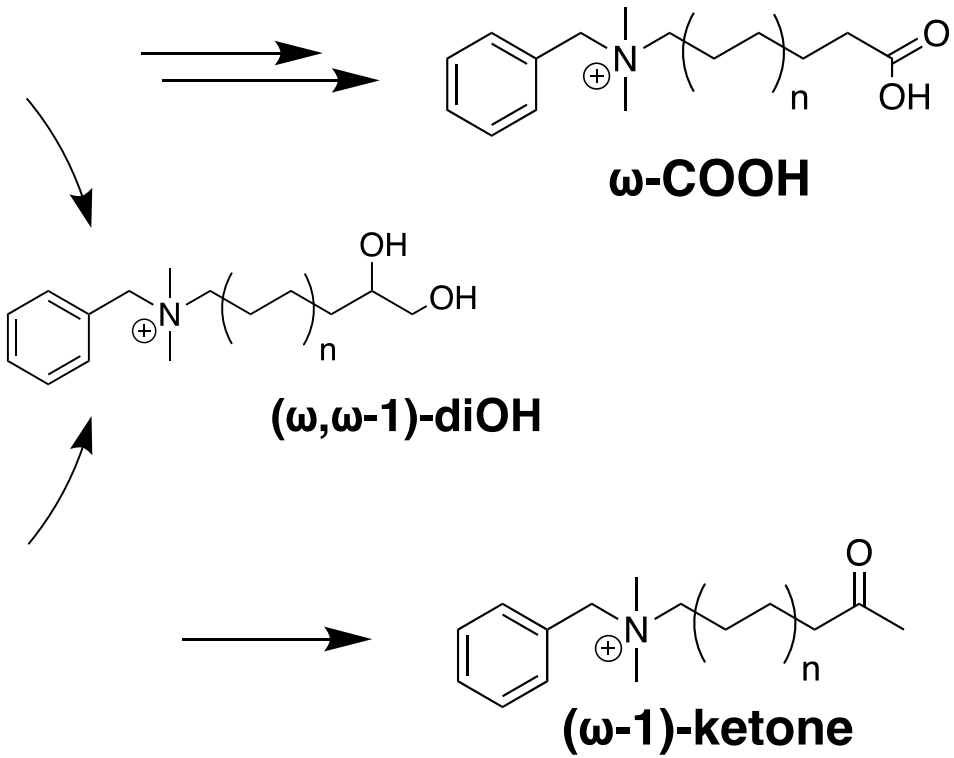
Animal

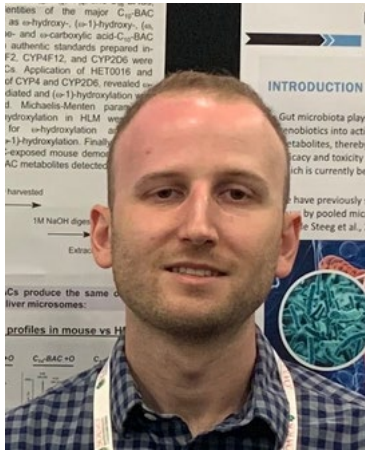
- Decreased reproduction

Microbe

- Antibiotic resistance

BACs Are Metabolized by CYPs



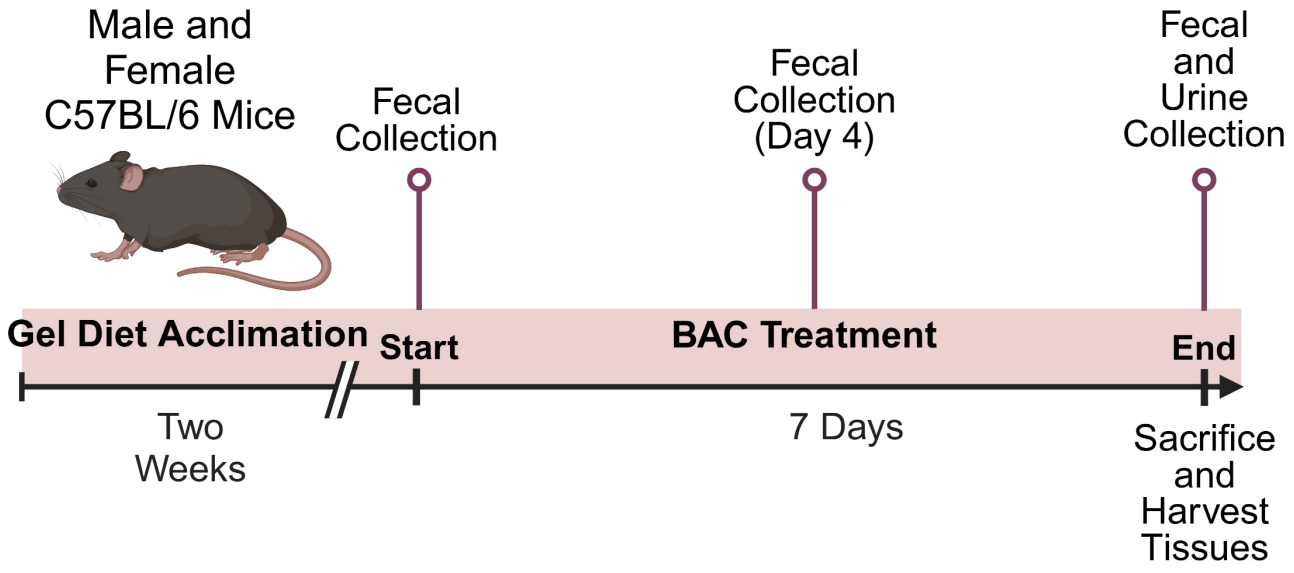


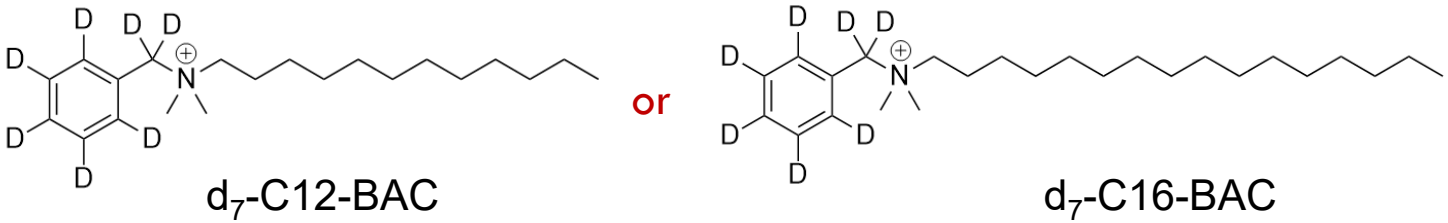
Ryan Seguin

Seguin, R. P., et al. (**2019**) *Chem. Res. Toxicol.*, *32*(12), 2466–2478.
Seguin, R., and Xu, L. (**2022**) *ASPET 2022 Annual Meeting*.
Lopez, V. A. *et al. Toxicol. Sci.* (**2024**) 202, 265–277.

BAC Distribution Among Organs

Mice Orally Exposed to BACs

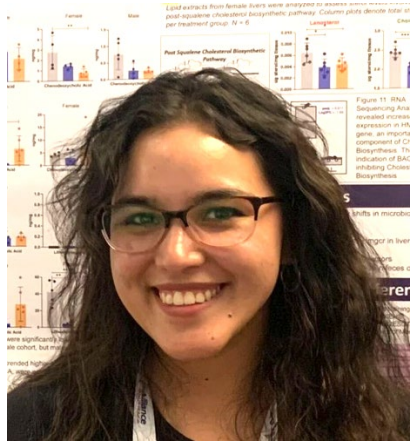




d_7-C12-BAC

d_7-C16-BAC

120 µg of each BAC/g/day



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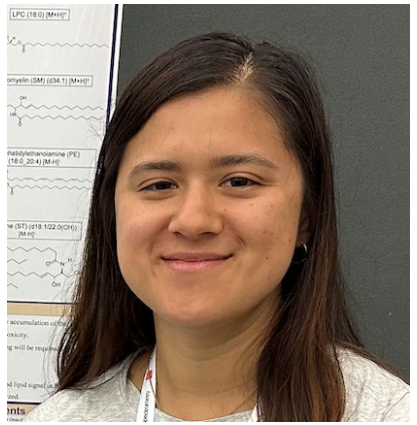
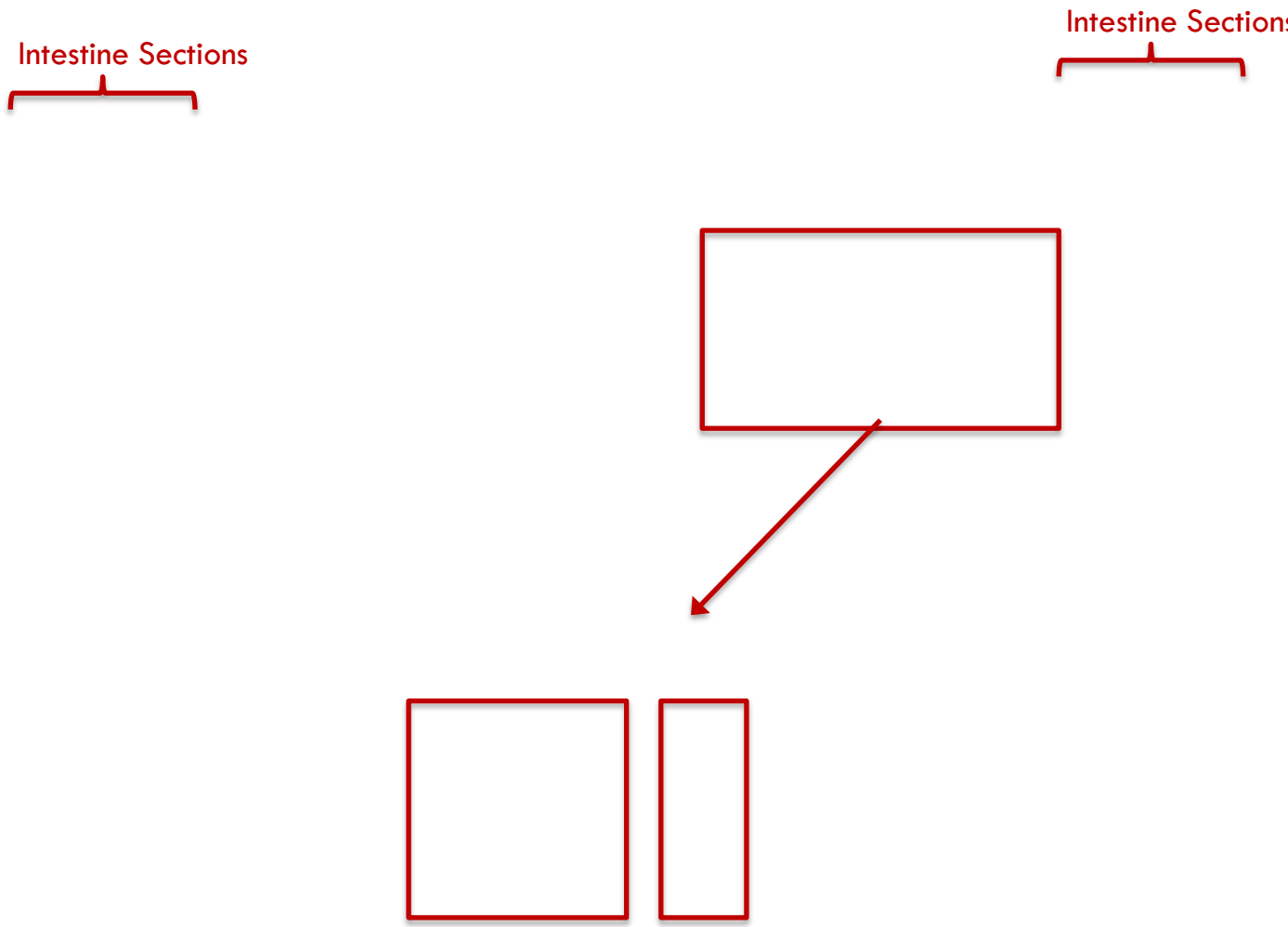


Marie Brzoska

Levels of Parent BACs in Tissues and Fluids



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Marie Brzoska

Parent to Metabolite Ratios in Tissues (Female)

Intestinal Sections

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Marie Brzoska

Levels of BACs in Feces

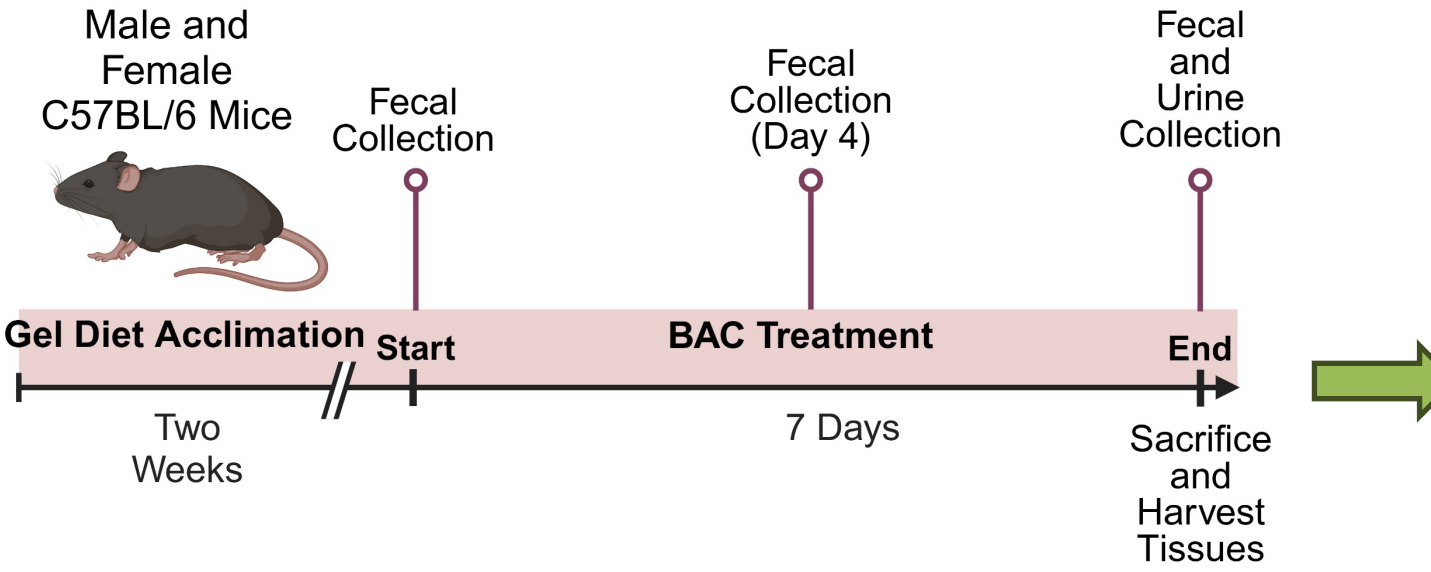
Parent
BACs:

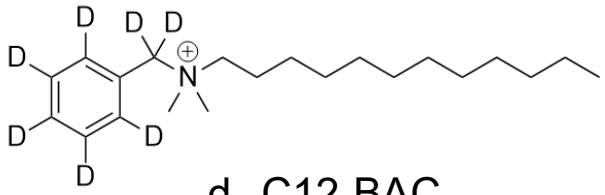
Vanessa Lopez
Marie Brzoska

$$\frac{Parent}{Metabolite}$$

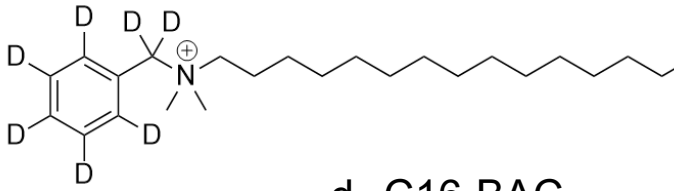
BACs are absorbed, metabolized, then excreted to the feces

Exposure Scheme



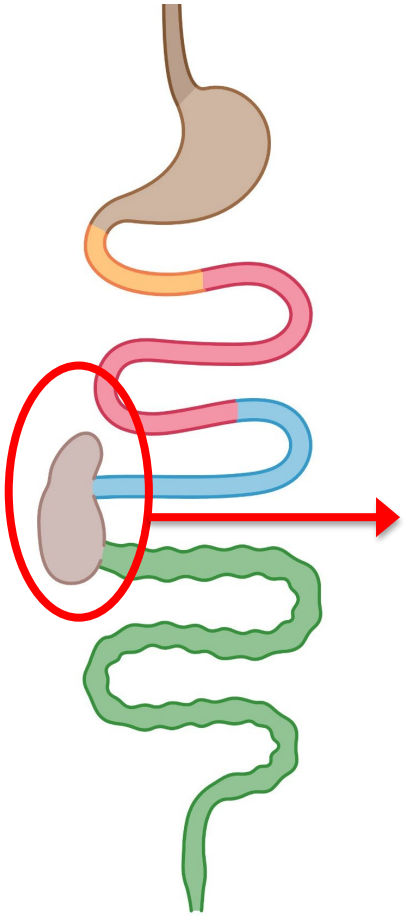


d_7-C12-BAC



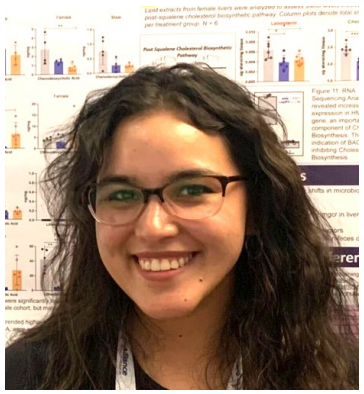
d_7-C16-BAC

120 µg of each BAC/g/day



Cecum intestinal content

16S rRNA sequencing



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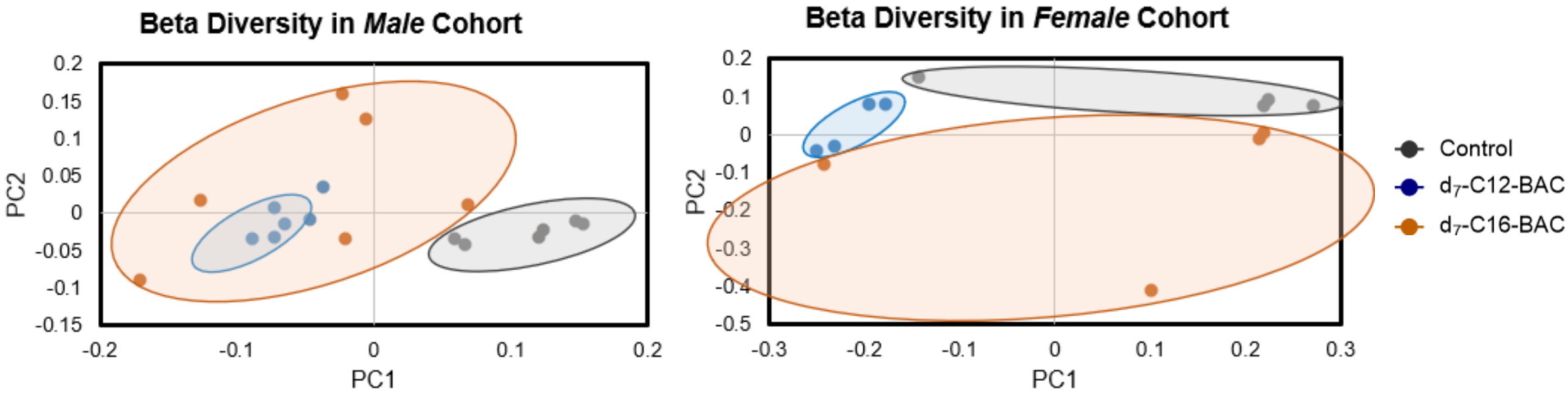
In collaboration with Julia Cui Lab

Lopez, V. A. *et al. Toxicol. Sci.* 202, 265–277 (**2024**).

BAC Exposure Led to Different Microbial Communities



Beta diversity measures the differences between different microbial communities

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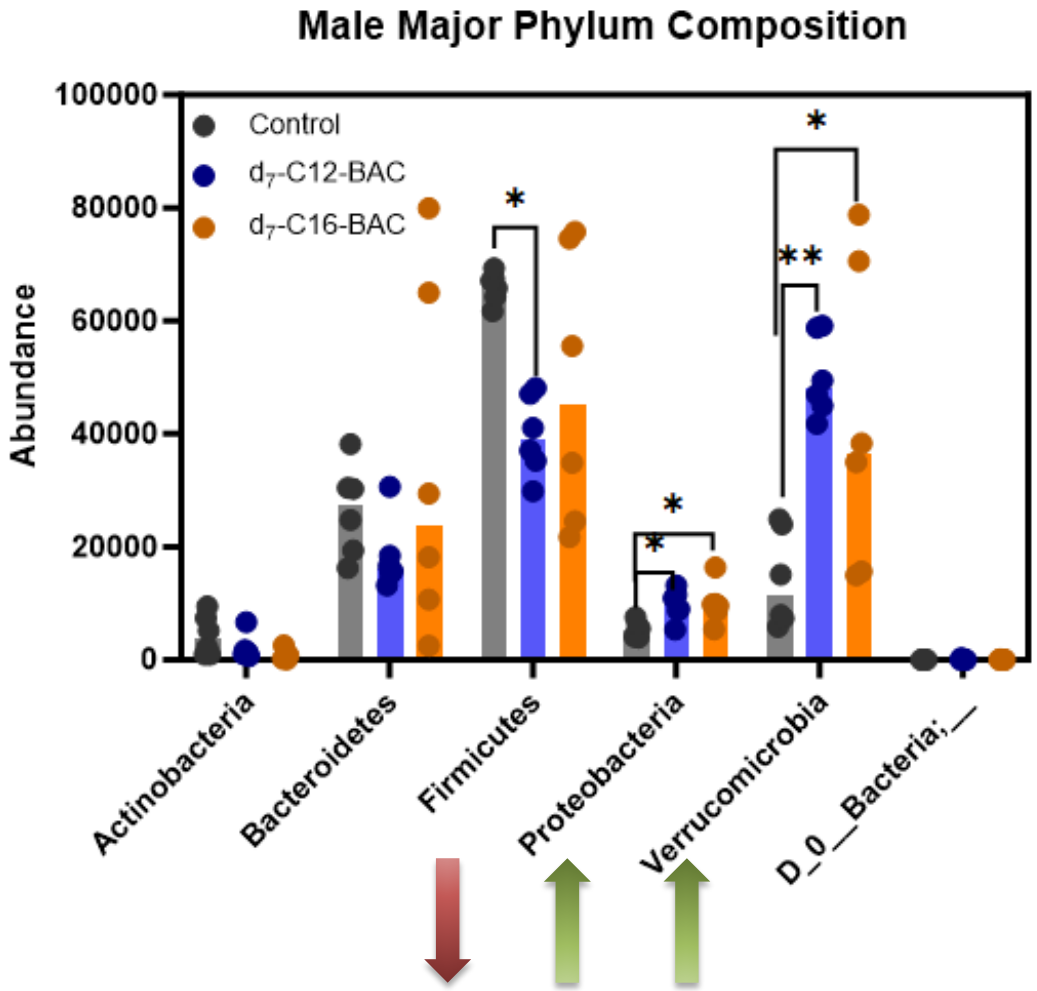
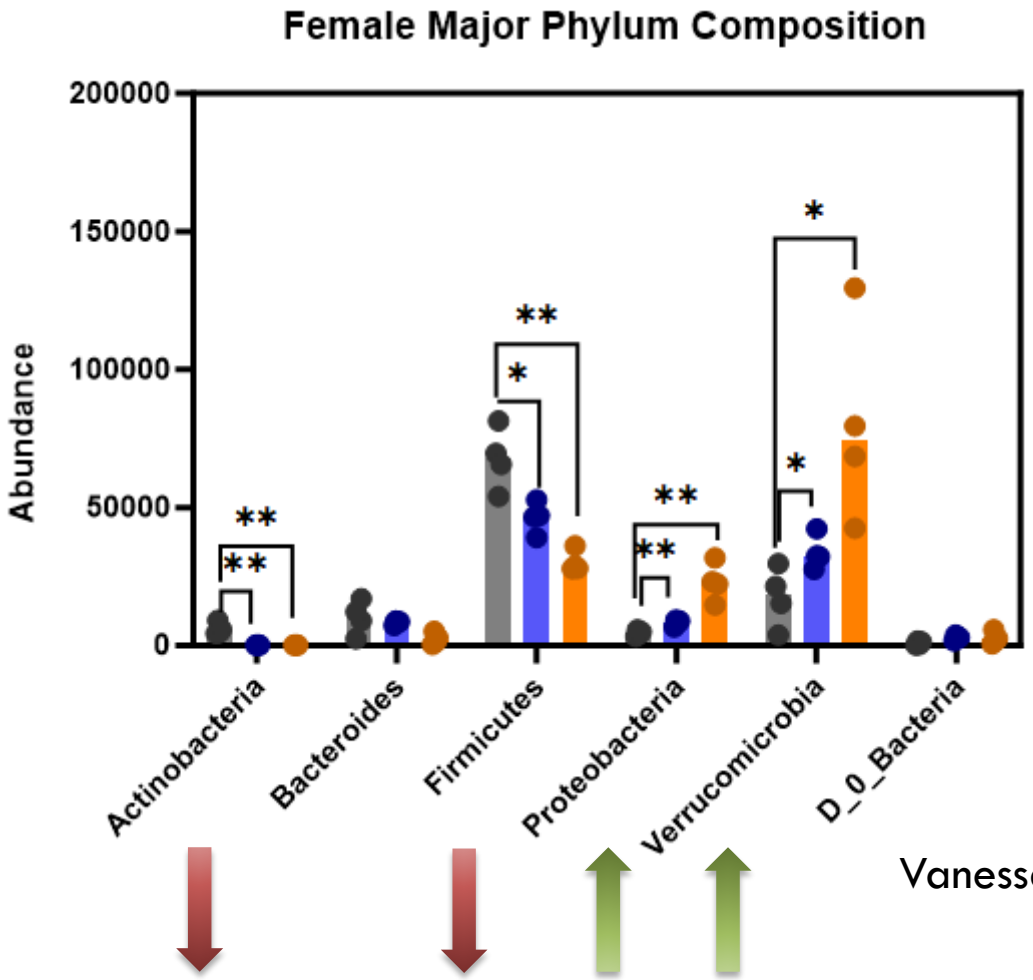
Compositional Changes at the Phylum Level





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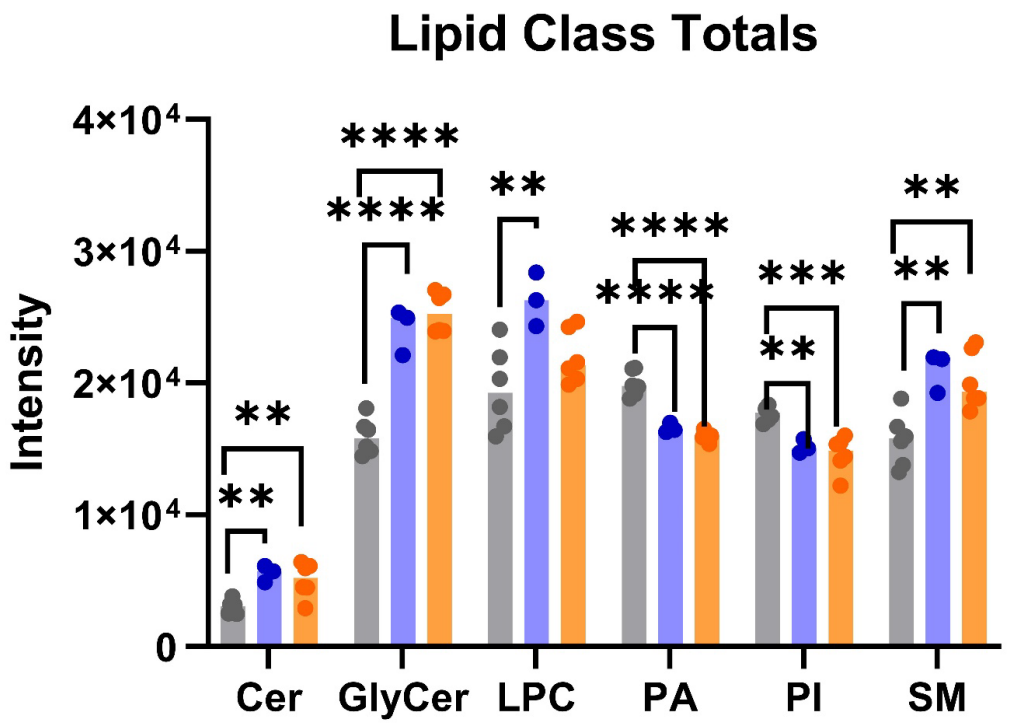
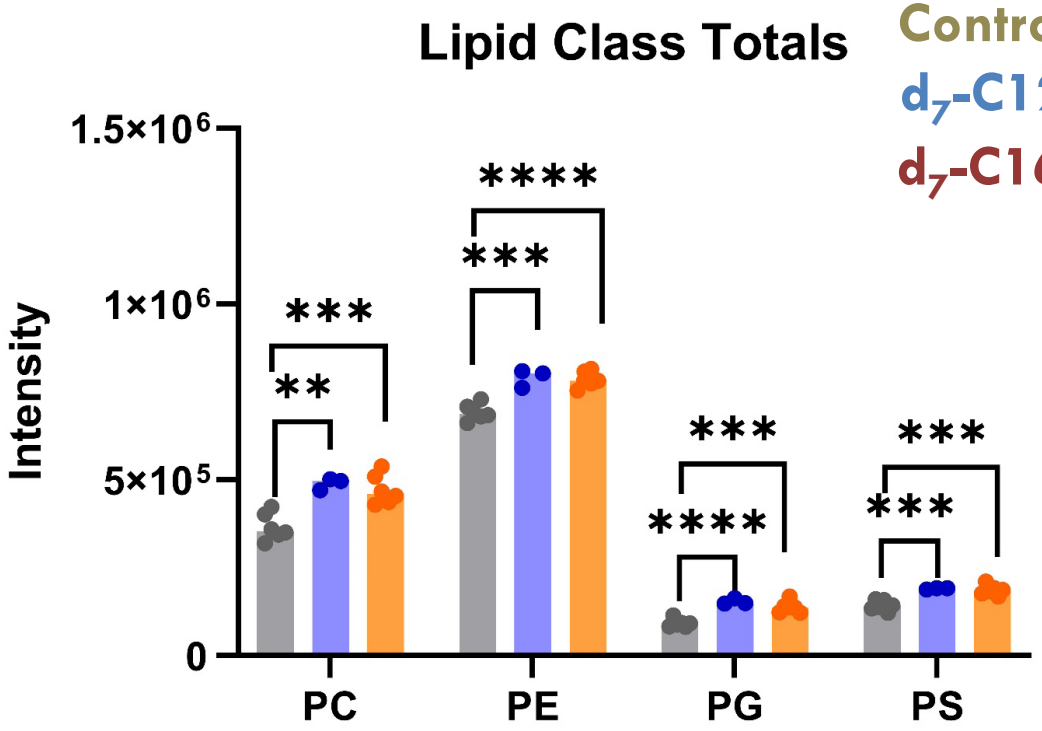
Lopez, V. A. *et al. Toxicol. Sci.* 202, 265–277 (**2024**).

Negative Mode Lipidomic Analysis revealed differential lipid class totals between Control and BAC treated mice

***Male* Cohort**





Ceramide (Cer)
Glucosylceramide (GlcCer)
Lysophosphatidylcholine (LPC)
Phosphatidic Acid (PA)
Phosphatidylcholine (PC)
Phosphatidylethanolamine (PE)
Phosphatidylglycerol (PG)
Phosphatidylinositol (PI)
Phosphatidylserine (PS)
Sphingomyelin (SM)