S12: Exploring Nutritional Psychiatric Guidelines for a Clinical Trial on Natural Products in Children with ADHD: A Focus on Biological Sample Collection

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Helfgott Research Institute National University of Natural Medicine

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Outline

- MADDY Study
- ISNPR Guidelines
- Implementation in the MADDY Study
- Blood Sampling: Plasma Immune Factor Analysis
- Stool Collection: Gut Microbiome & Metabolome Analysis
- Urine Collection: Glyphosate Analysis

ISNPR: International Society for Nutritional Psychiatry
MADDY: Micronutrients for ADHD in Youth

No financial conflicts to declare

- *Studies were funded by unrestricted philanthropic gifts*
- *Manufacturers provided the formulas, but had no input in the studies themselves or their reporting*
- *We have no commercial interest in any company or product.*

Micronutrients for ADHD in Youth Study (MADDY)

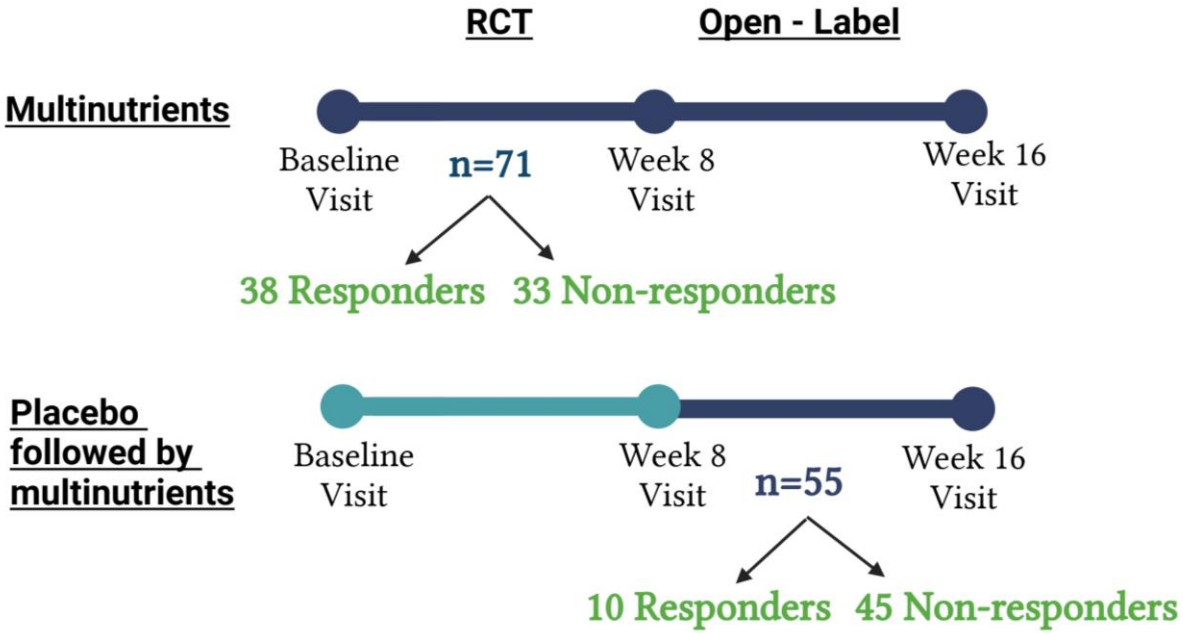
THE OHIO STATE UNIVERSITY

Methods of MADDY (N=126)

- Multinutrients (or micronutrients)
  - Essential vitamins, minerals, amino acids and antioxidants
- Double Blind, RCT
- Age: 6-12 year olds with ADHD
- N=126
- Collected biological samples

Johnstone et al. *JAACAP* 2022

Study Design (N=126)



Johnstone et al. *JAACAP* 2022

Study Guidelines

International Society for Nutritional Psychiatry

NIH K23, Associate Professor
Jeni Johnstone, PhD, MA

International Society for Nutritional Psychiatry Research (ISNPR)

ISNPR Mission Statement:

To advance scientifically rigorous research and the pre-clinical, clinical, and public health implementation of nutritional approaches to help prevent and treat mental health disorders and their comorbidities.

Marx et al., British Journal of Nutrition (2024) Methodological and reporting recommendations for clinical trials in Nutritional Psychiatry: Guidelines from the International Society for Nutritional Psychiatry Research

http://www.isnpr.org/

Delphi Process

- **Step 1: Expert Panel** – A group of experts or knowledgeable people in a specific field are selected. These could be clinicians, researchers, or specialists with relevant experience.
- **Step 2: Anonymous Questionnaires** – Each expert is asked to answer a series of questions or give their opinion on a particular issue. The goal is to gather their individual views without influence from others.
- **Step 3: Round 1** – Experts provide their initial responses. These answers might be broad or varied.
- **Step 4: Summary Feedback** – After the first round, the responses are summarized. The feedback is shared with the group, showing trends, common points, or differences. Experts can see how their opinions compare to others.
- **Step 5: Round 2 (and sometimes more)** – Based on the feedback, experts are asked to reconsider their answers or adjust them in light of the group's collective input. This process can continue over several rounds.
- **Step 6: Consensus** – Over the rounds, experts refine their opinions, and the group begins to reach a consensus on the best approach or solution to the issue.

Clinical Trial Guidelines

- Delphi Process – 18 researchers
- 61 recommendations;
  - 49 for trial design
  - 5 for trial reporting
  - 7 for future research

Marx et al., 2024 British J of Nutrition

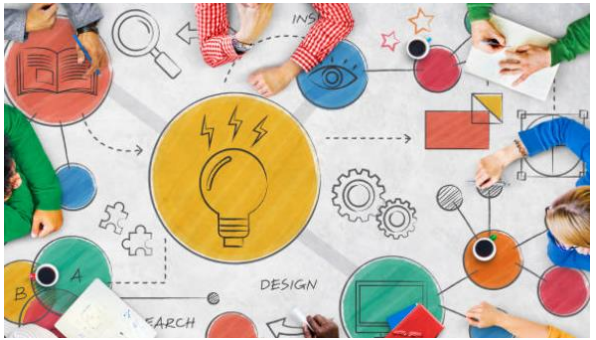
Recommendations

Recommendations: Study Team

- Multidisciplinary team
- Engage individuals with lived experience
- Registered dieticians for dietary studies

Recommendations: Trial Design

- Consider participant burden, potential adherence barriers
- Predefine adherence assessment methods
- Multimodal data collection methods
- Mitigate expectancy bias
- Ensure adequate power
- Specify sufficient dose / duration
- Account for seasonal variation



Recommendations: Participants

- Use recruitment strategies to enhance sample generalizability to broad population
- For dietary studies, establish clear protocols for participants with eating disorders
- Enroll participants with sufficient "disorder" to avoid potential floor effect
- Use accepted cut-offs for eligibility criteria
- Account for comorbidities

Recommendations: Interventions

- Manualize diet or behavioral interventions; test for fidelity / adherence
- Dietary interventions – provide guidance on food safety, hygiene, storage, preparation, especially if introducing unfamiliar foods
- Ensure tailored delivery to the population
- In rural or remote areas – ensure ingredient availability, and/or transport is available

Recommendations: Placebo or Comparator

- Justify and explain comparison condition
- Use a placebo, not a waitlist
- Match expectancy and engagement in the control
- Consider non-inferiority trial designs for current treatments

Recommendations: Outcomes

- Use self- and clinician-rated outcomes
- Measure at multiple timepoints; EMA
- Use total scores and individual symptom changes
- Include relevant biological samples
- Consider subgroup analyses
- Measure safety

Recommendations: Reporting Outcomes

- Measure dose, frequency, composition, timing of the intervention
- Define, a priori, primary outcomes
- Clearly delineate extent of industry involvement
- Authors declare conflicts of interest / bias
- Make trial protocols publicly available

Recommendations: Future Research

- Consider diverse research settings: inpatient and outpatient, different healthcare environments
- Test over-the-counter formulas or popular diets (e.g.ketogenic) that have limited clinical data
- Conduct scaleable research (virtual, group-based)
- Examine treatment effect moderators
- Include under-researched populations

Summary

- Build a diverse research team
- Use these guidelines

International Society for Nutritional Psychiatry Research (ISNPR)

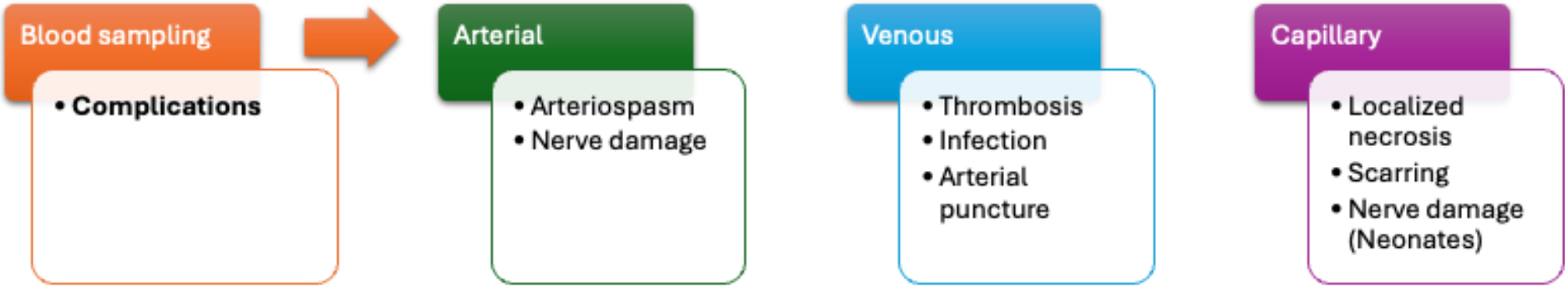
Blood Sampling

Plasma Immune Factor Analysis

NIH T32 Post-doctoral researcher
Taryn Machingo, ND, MS

Blood sample collection

- Blood samples are the most commonly collected bio-specimens
- Anticoagulants
  - EDTA, heparin, citrate
    - Complete blood count
- Serum separation
  - Comprehensive metabolic panel
- Storage
  - 2 to 8 degrees Celsius or –20 to –80 degrees Celsius
  - Protected from direct light exposure

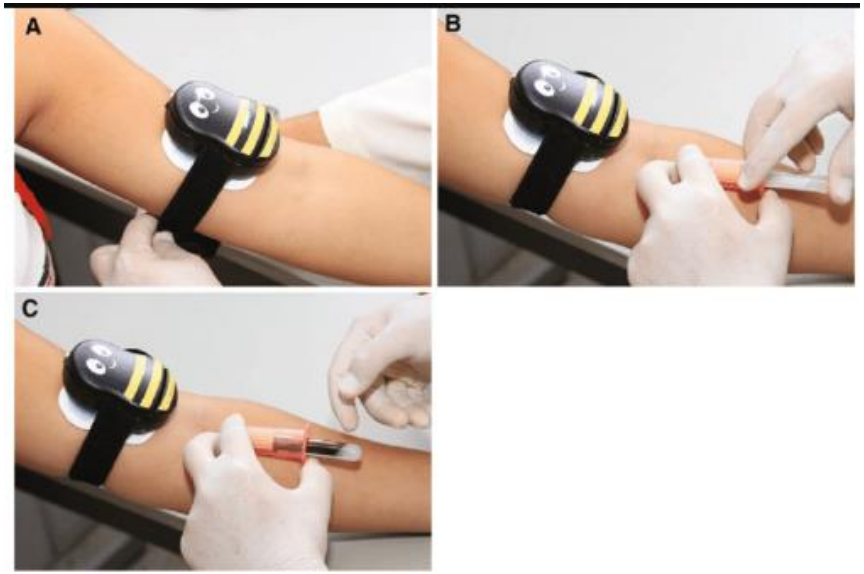


Vasovagal response
Hematoma

Pain & anxiety relief during specimen collection

Application of external cold and vibration via Buzzy®

- Decreased perceived pain and anxiety
- Few clinically significant differences between samples that did and did not use Buzzy®
- Alternative use as distraction



Use of upper arm based capillary blood collection devices

Patients prefer this collection method to traditional venipuncture

Concordance between venous and capillary samples was high

Baseline association of Suicidality with pro- and anti-inflammatory immune factors in children with ADHD

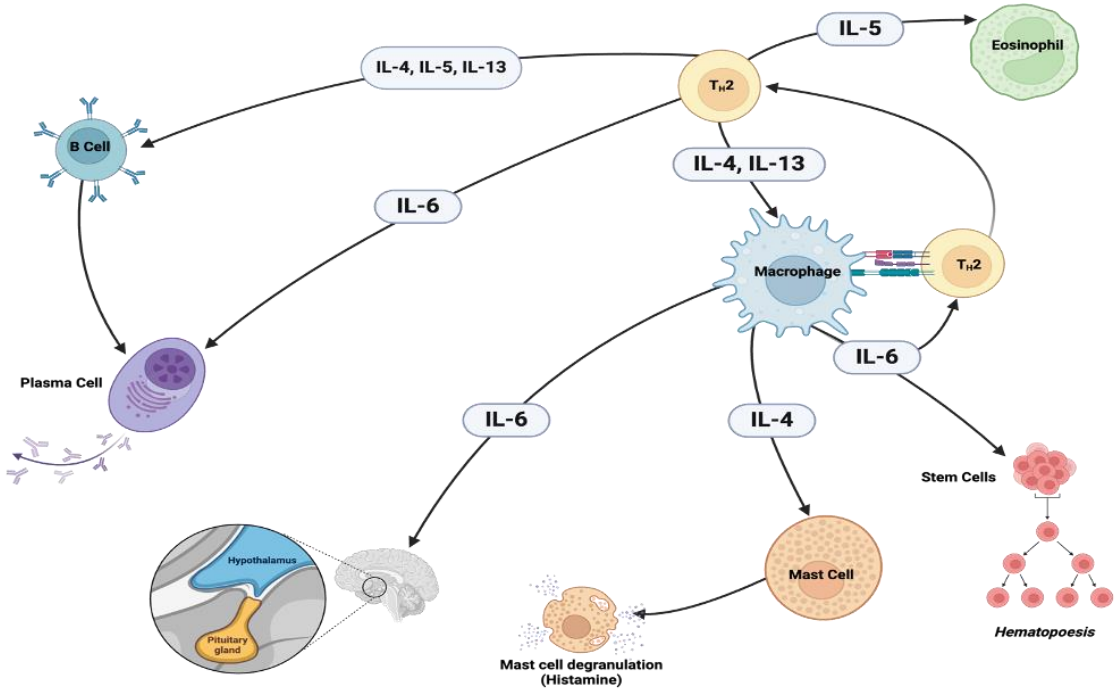


Figure 1. Inflammation can be assessed through an examination of immune factors. Multiple inflammatory immune factors are elevated in individuals with ADHD compared to individuals without.

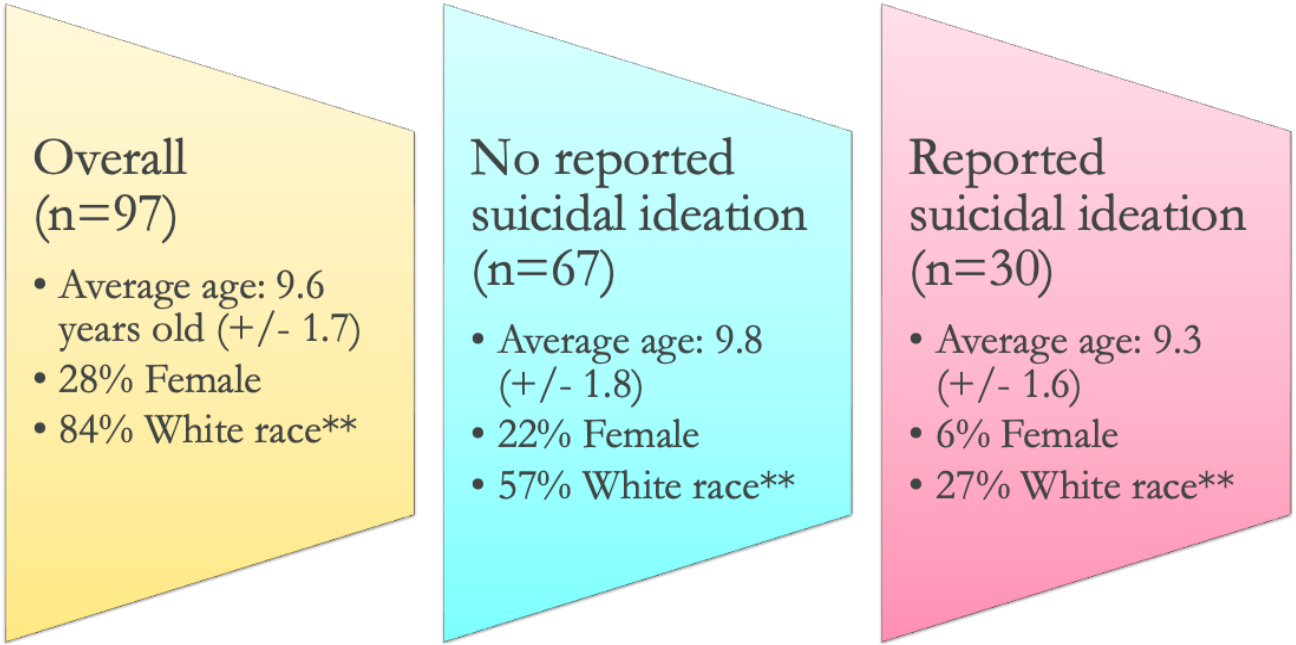
Baseline association of Suicidality with pro- and anti-inflammatory immune factors in children with ADHD

Methods:

- 19 immune factors were analyzed
  - Known role in psychiatric pathology
- Plasma immune factor concentrations were measured using multiplex assays on a Luminex LX-200 instrument
- Panels
  - Cancer Multiplex Assay
    - HAGP1MAG-12
- leptin, human growth factor (HGF), and vascular endothelial growth factor-A (VEGF-A)
  - Immunology Multiplex Assay
    - HCYTOMAG-60K

Baseline association of Suicidality with pro- and anti-inflammatory immune factors in children with ADHD

Results:



**Participants were allowed to select all race and ethnicity categories that apply. Numbers may sum to larger than sample size because participants selected multiple answers.

Table 2: Mann-Whitney-U test results comparing median immune factor levels between participant who did and did not endorse suicidality

Cytokine	No SI (n=67)	Reported SI (n=30)	p-value
Eotaxin	68.5	78.8	0.09
GCSF	11.3	21.3	0.08
HGF	123.8	133.5	0.15
IL-12-p-70	4.9	5.0	0.89
IL-13	48.7	21.6	0.17
IL-15	10.5	7.6	0.34
IL-17a	4.0	4.0	0.84
IL-2	2.3	2.3	0.16
IL-4	256.2	61.2	0.13
IL-5	7.6	4.9	0.21
IL-6	33.1	10.5	0.11
IP-10	357.4	355.4	0.92
Leptin	6165.0	6825.0	0.76
MCP-1	216.0	208.4	0.88
MIP-1α	11.3	11.3	0.91
MIP-1β	17.3	18.9	0.08
MDC	701.9	694.1	0.95
TNF-α	17.0	17.9	0.92
VEGFA	72.7	92.9	0.88

GCSF, Granulocyte colony-stimulating factor; HGF, Hepatocyte growth factor; IL, Interleukin; IP, Interferon gamma-induced protein; MCP, Monocyte chemoattractant protein; MIP, Macrophage Inflammatory Proteins; MDC, Myeloid dendritic cell; TNF, Tumor necrosis factor; VEGF, Vascular endothelial growth factor

Key Findings:

Several immune factors (eotaxin, GCSF, MIP-1β) showed a trend toward statistical significance, suggesting a possible difference between groups that warrants further investigation. This would require a larger sample size and a more age-appropriate screening tool for suicidal ideation.

Stool Collection

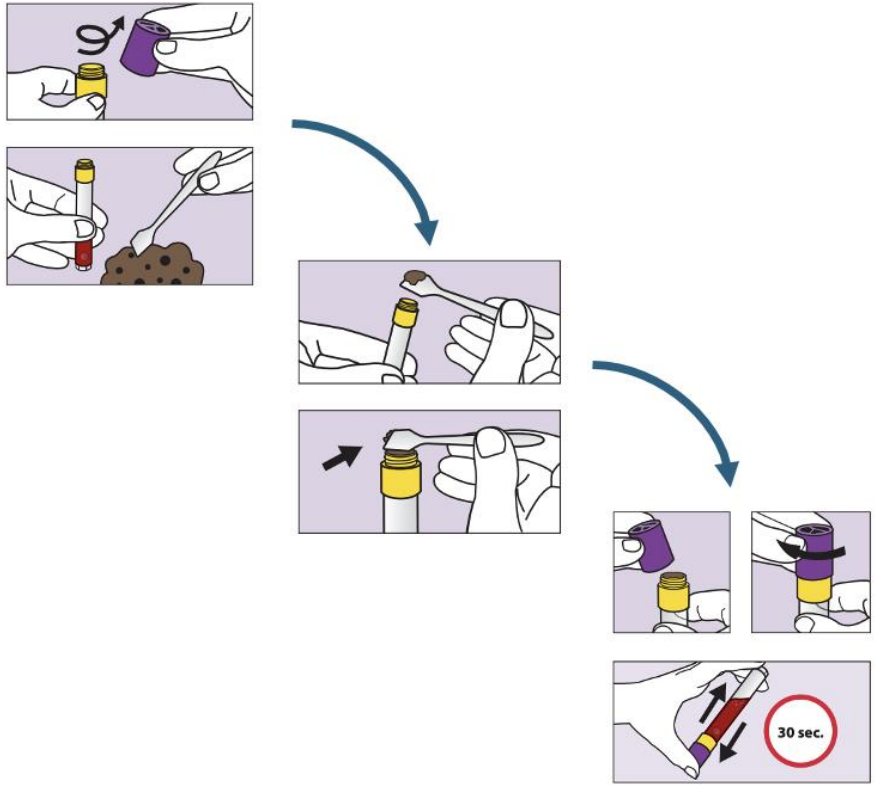
Gut microbiome and metabolomic response to micronutrients in children with ADHD

Sample Collection

- Stool samples collected using OMNIgene-gut collection kits at home
  - Kits offer stabilization of fecal DNA and RNA at room temperature.
- 3 timepoints
  - Baseline
  - Week 8 (RCT end)
  - Week 16 (open label extension end)

Procedure

- Stool samples collected at home in all MADDY participants
- Instructed to place sample in test tube.
- Tubes shaken to stabilize.
- Samples returned at next visit (within 1 month) and immediately stored at -80°C until analysis.
  - Stability – 30 days for metabolites and 60 days for microbiome.



Collection instructions from DNA Genotek Inc.

Microbiota data generation and processing

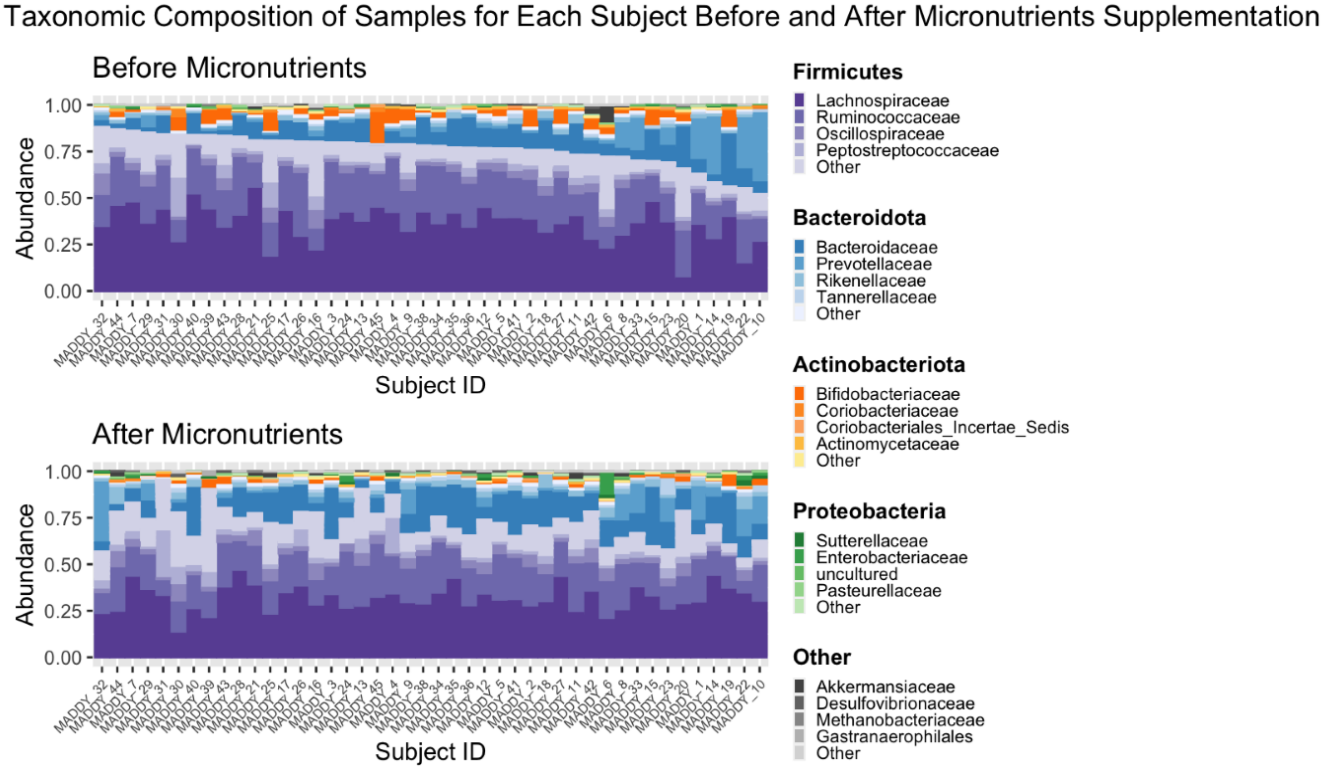
Sub-cohort analysis of fecal samples (n=50)

Samples sent to Pacific Northwest National Laboratories for DNA extraction 16s rRNA gene sequencing and raw data processing.

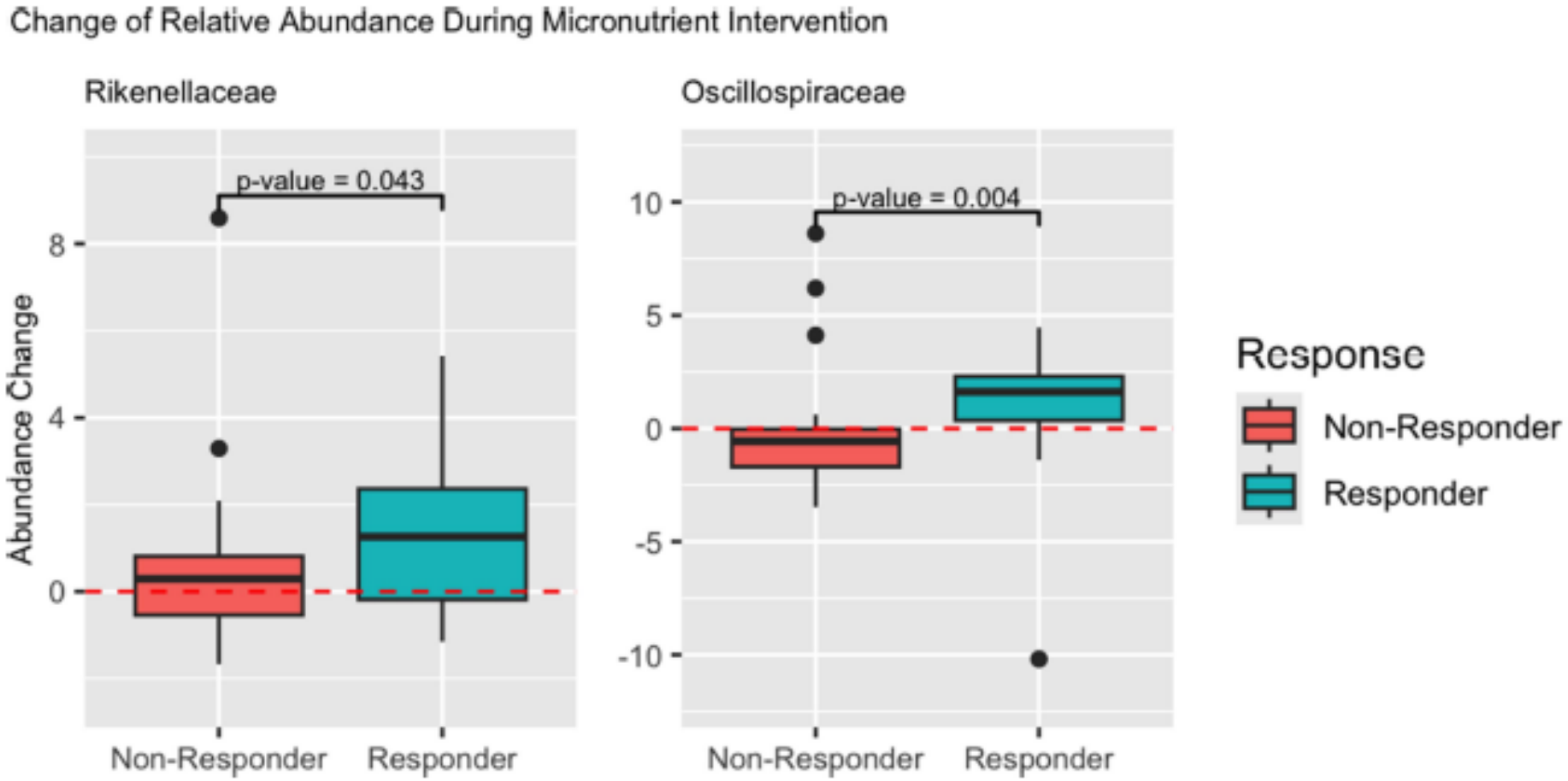
DNA extraction performed

Sequencing performed on an Illumina MiSeq.

Microbiome Results



Ast et al. *Gut Microbes* 2025
Stevens et al. *Scientific Reports* 2019



Ast et al. *Gut Microbes* 2025

Metabolite identification and analysis

Data processing and analysis performed by Pacific Northwest National Laboratories

Metabolite content from was analyzed by liquid chromatography--mass spectrometry (LC-MS).

3 datasets: C18 negative mode, C18 positive mode, and HILIC mode.

Data analyzed for changes in abundance.

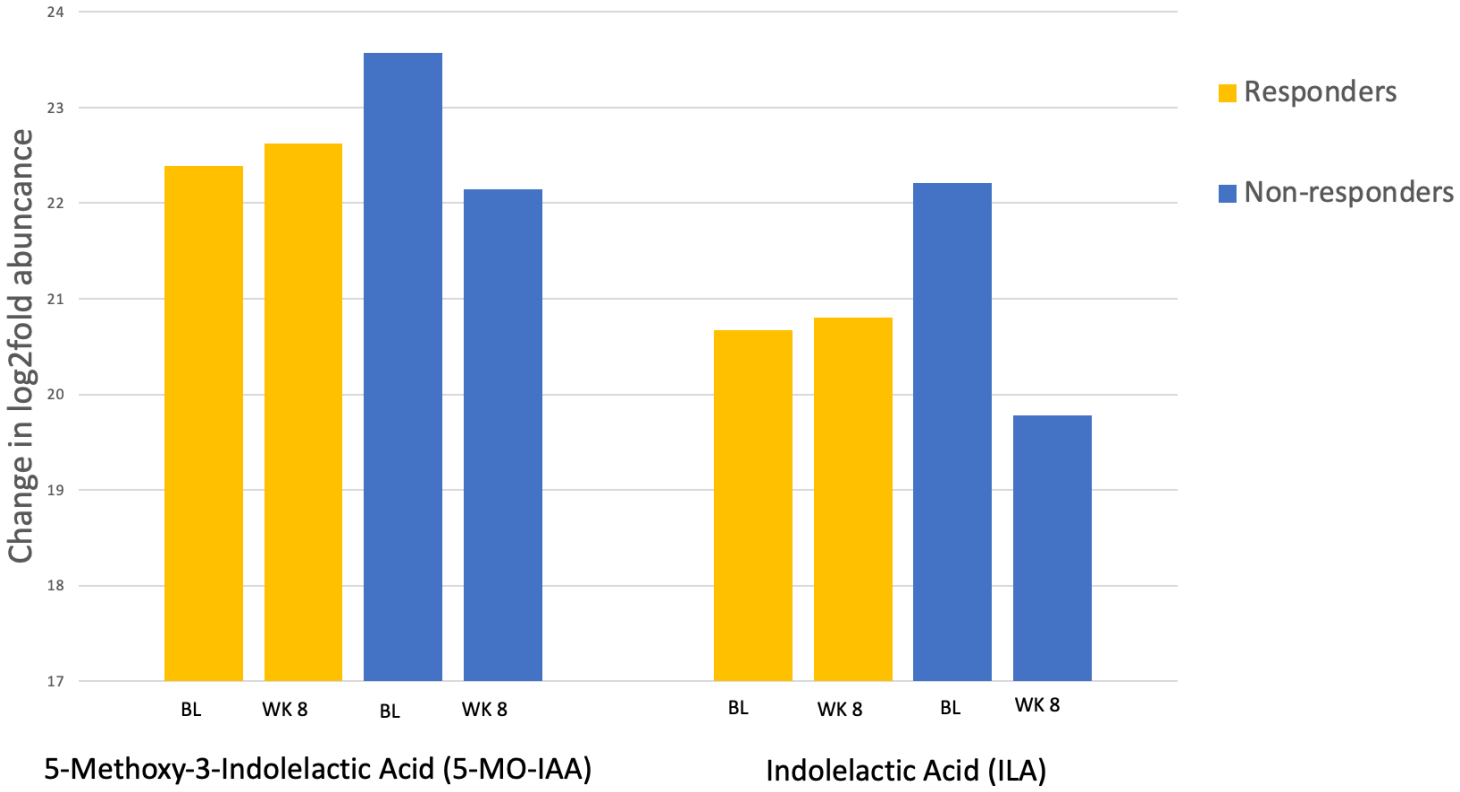
Metabolite Analysis

Products of microbial metabolism

Functionally active

Neurotransmitters, cell wall components, immune modulators, etc.

Change in Tryptophan Metabolite Abundance



Urine Collection

Urinary Glyphosate Analysis

NIH T32 Post-doctoral researcher
Hayleigh Ast, ND

Liquid Urine

OR

Dried Urine

Images Created with BioRender and Canva AI

Liquid Urine

- Collection
  - Pee in a cup
  - Aliquoting samples
  - Freezing (-80°C) and storage
- Validity
  - Gold Standard

Dried Urine

- Collection
  - Similar to liquid collection
  - Easy to transport
  - Cheaper to store
  - Stability at room temperature
    - Long term storage -80°C
- Validity
  - No independent test for efficacy found

Newman and Curran BMC Journal 2020 & 2021

Dried Biosampling

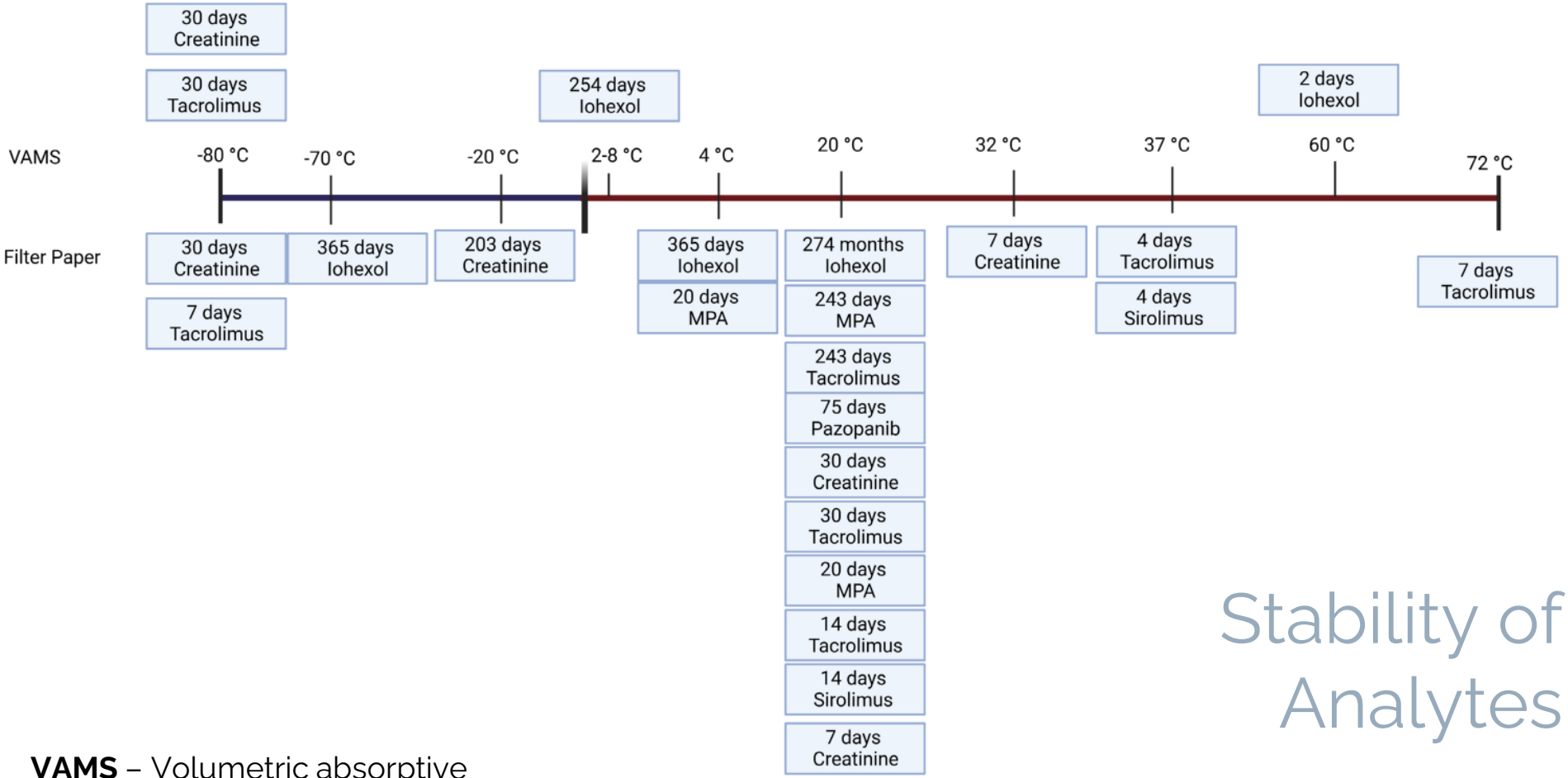
Lamond et al. Journal of Clinical Lab. Analysis 2024
Protti et al. Analytica Chimica Acta 2019

Pro

- Cost saving
- 24 hour urine collection
- Overcome participation bias
- At home test
  - Remote participants
  - Conduct test on your own time
    - During flares
    - No time off work

Con

- Mixed results for **sample recovery**
- Sampling devices poor quality by 32% compared to filter paper 6%
- Mixed methodology



Stability of Analytes

VAMS – Volumetric absorptive microsampling devices

What is best for researchers?

- Lab partnership
- Type of urine collection required
  - 24 hour vs single time point
- Cost of tests
- Pediatric population
  - (n=28) 50% preferred dried blood; 42% blood draw; 8% uncertain
- Remote study
  - Large studies, easy transport
  - Comparable to liquid urine

Martial et al. Ther Drug Monit 2017
Morohashi et al. Pediatrics International 2021
Newman and Curran BMC Journal 2020 & 2021

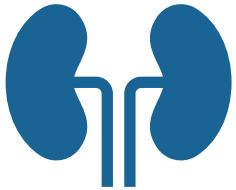
Glyphosate - MADDY Study

- Herbicide: kills unwanted plants
- Residues on food which lead to low dose long term exposure (LDLT)
- Elevated levels in children (CDC 2022)
- Unknown impact of LDLT in children

Castilo et al. International Journal of Molecular Sciences 2022
Walsh et al. Gut Microbes 2023

Images Created with BioRender

Glyphosate

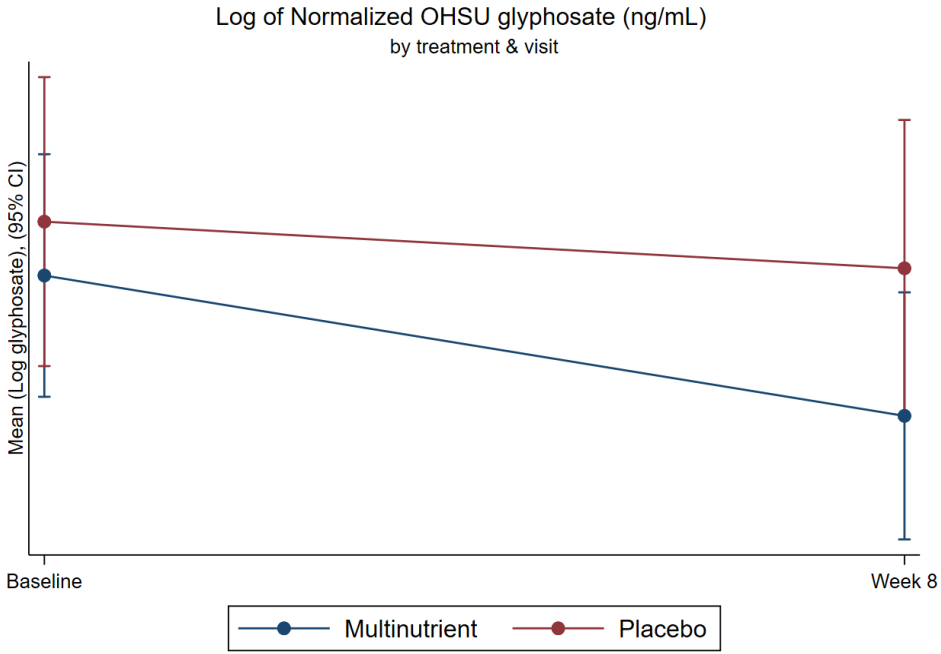


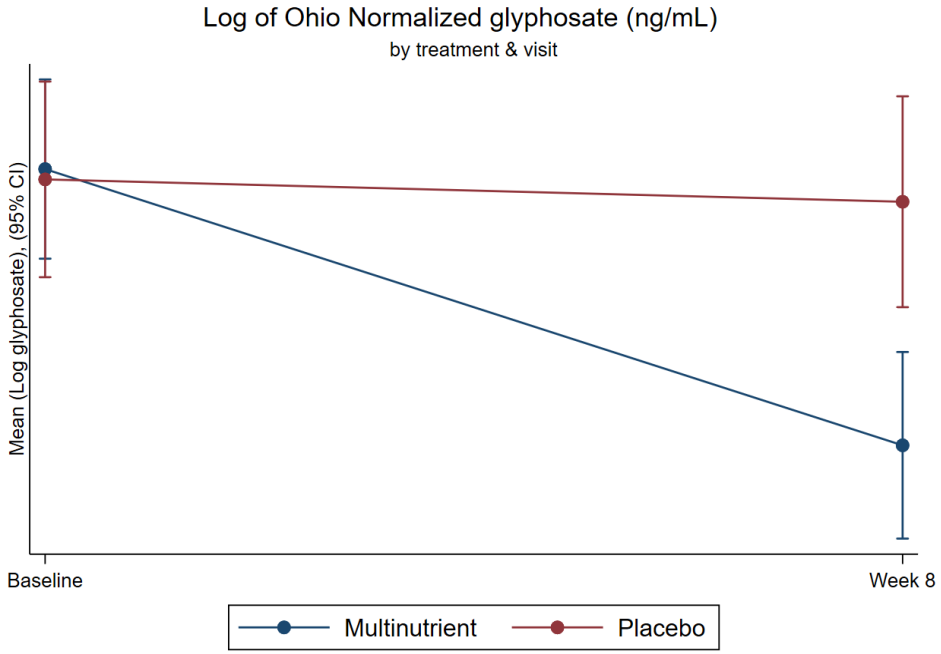
24-hour urine collection is best

Morning collection is second best

Toxicology Profile for Glyphosate Ch. 3, 2020

Results


Log of Normalized OHSU glyphosate (ng/mL)
by treatment & visit
Mean (Log glyphosate), (95% CI)
Baseline
Week 8
Multinutrient
Placebo


Log of Ohio Normalized glyphosate (ng/mL)
by treatment & visit
Mean (Log glyphosate), (95% CI)
Baseline
Week 8
Multinutrient
Placebo

Liquid Urine

- Gold Standard
- Reliable Methods
- Burdensome to store and transport

OR

Dried Urine

- Convenient for family and researchers
- Mixed methods/reliability

Images Created with BioRender and Canva AI

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Building Research ACROSS
Inter-Disciplinary Gaps

OHSU

the waterloo foundation

Thank You

SNACK Lab at OHSU

Jeni Johnstone, PhD

Hayleigh Ast, ND

Taryn Machingo, ND, MS

Lydia Norby-Adams, BA

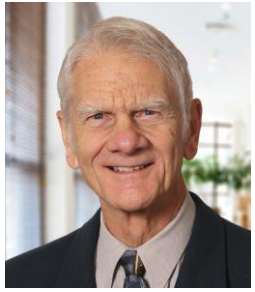
Alisha Bruton, MS, ND

Priya Srikanth, MS

Not Pictured, but equally appreciated: The Pacific Northwest National Laboratory – Ryan McClure, PhD and Sneha Couvillion, PhD; University of Southampton - Jon Swann, PhD; OHSU Volunteer- Alanna Walsh, ND; Health Research Institute - John Fagan, PhD

University of Lethbridge: Brenda Leung, ND

Ohio State University: Eugene L. Arnold, MD, Med, Irene Hatsu, PhD, Lisa Robinette, MS; Not Pictured: Chris Zhu, PhD, Shiqi Zhang, PhD, Xinru Pang



OHSU: Dept of Medical Informatics and Clinical Epidemiology: Lisa Karstens, PhD, Matthew Hammer, MS

Discussion

References:

SNACK Lab: