

# 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



**2025  
International  
Congress**  
on Integrative Medicine & Health



# 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.*

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# Micronutrients for ADHD in Youth Study (MADDY)

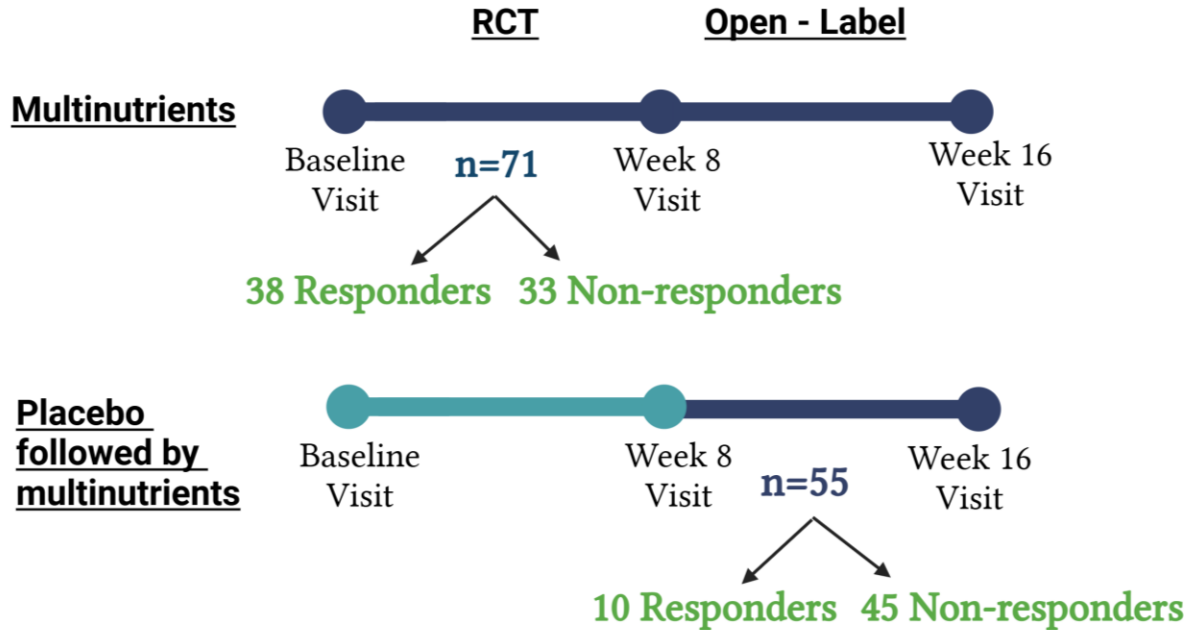


# Methods of MADDY (N=126)

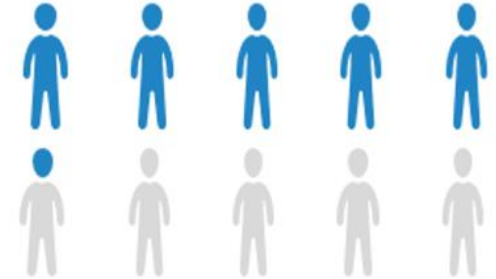
- ▷ Multinutrients (or micronutrients)
  - Essential vitamins, minerals, amino acids and antioxidants
- ▷ Double Blind, RCT
- ▷ Age: 6-12 year olds with ADHD
- ▷ N=126
- ▷ Collected biological samples



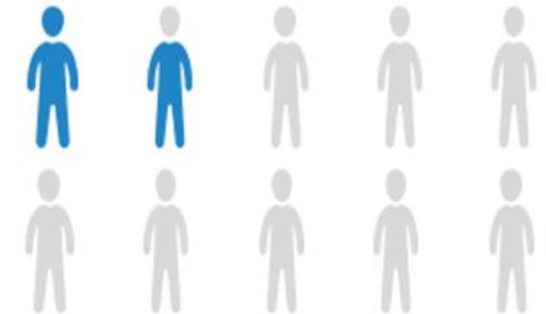
# Study Design (N=126)



Micronutrient Group: **54%**



Placebo Group: **18%**



- = Multinutrient Intervention
- = Placebo Intervention

# Study Guidelines

International Society for Nutritional Psychiatry  
Research

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.*



# Delphi Process

- ▷ **Step 1: Expert Panel** – Knowledgeable people in a specific field; could be clinicians, researchers, or specialists with relevant experience.
- ▷ **Step 2: Anonymous Questionnaires** – Each expert answers a series of questions or provides 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**.
- ▷ **Step 4: Summary Feedback** – After the first round, the responses are summarized and 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 group discussion, 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

- 18 ISNPR researchers – Delphi process
- 61 Recommendations
- 8 Key Areas

# Recommendations: Study Team

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



# Recommendations: Trial Design

- ▶ Consider participant burden, potential adherence barriers
- ▶ Predefine adherence assessment methods
- ▶ Consider multimodal data collection method
- ▶ Mitigate expectancy bias
- ▶ Ensure adequate power
- ▶ Specify sufficient dose/duration



# Recommendations: Participants

- ▶ Use recruitment strategies to enhance sample generalizability
- ▶ Account for comorbidities
- ▶ Enroll participants with sufficient "disorder" to avoid potential floor effect
- ▶ Use accepted cut-offs for eligibility criteria



# Recommendations: Interventions

- ▶ Manualize the intervention; document fidelity or adherence
- ▶ Consider safety guidelines
- ▶ Ensure tailored delivery to the population
- ▶ In rural or remote areas – ensure ingredient availability, transport is available



# Recommendations: Comparator

- ▷ Use a placebo, not a waitlist
- ▷ Justify and explain comparison condition
- ▷ 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
- ▷ Contextualize the change





# Recommendations: Reporting Outcomes

- ▷ Define, a priori, primary outcome(s)
- ▷ Measure dose, frequency, composition of the intervention
- ▷ 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 environments (e.g. schools)
- ▶ Test over-the-counter formulas or popular diets (e.g. ketogenic) that have limited clinical data
- ▶ Conduct scalable research (virtual, group-based)
- ▶ Examine treatment effect moderators



# Summary

Lots to think about before starting a study.

**Join us!**

The 5th International Society for Nutritional  
Psychiatry Research Conference

October 18th-19th 2025, Taipei, Taiwan

[www.isnprconf.org](http://www.isnprconf.org)



# 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

Blood sampling



• **Complications**

Arterial

- Arteriospasm
- Nerve damage

Venous

- Thrombosis
- Infection
- Arterial puncture

Capillary

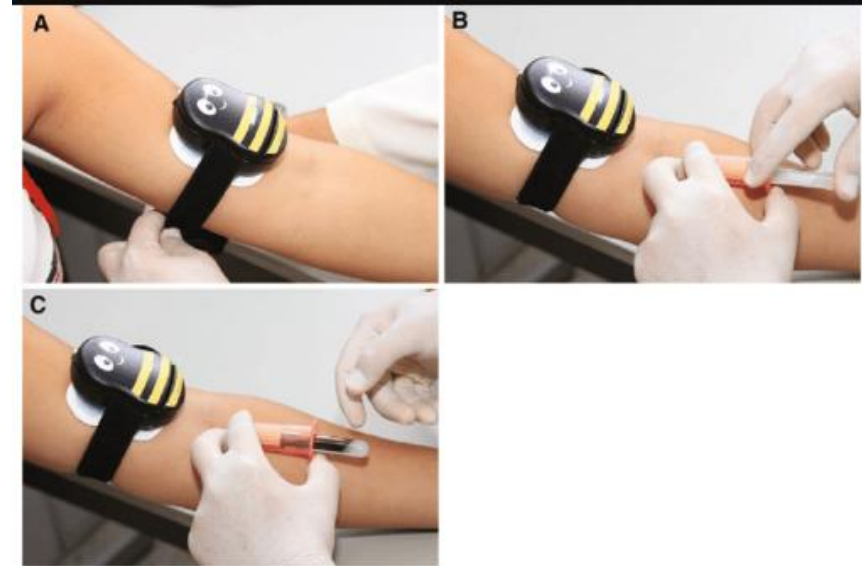
- Localized necrosis
- Scarring
- Nerve damage (Neonates)

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

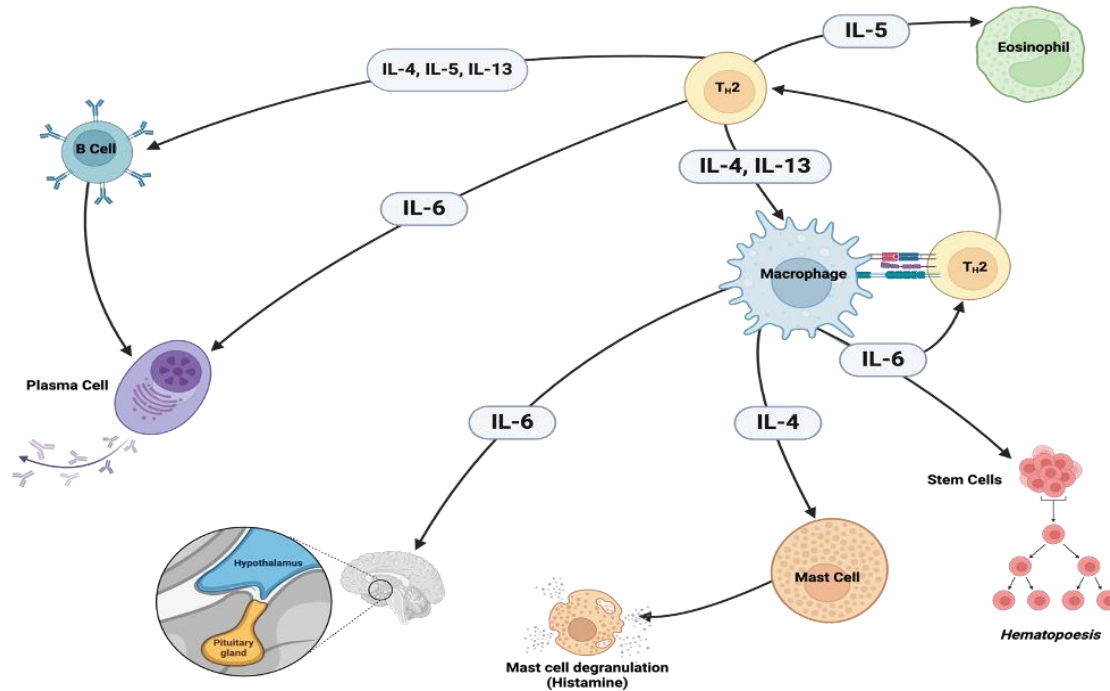


Figure 1. Inflammation can be assessed through an examination of immune factors. Multiple inflammatory immune factors are elevated in individuals with ADHD compared to individuals without.

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

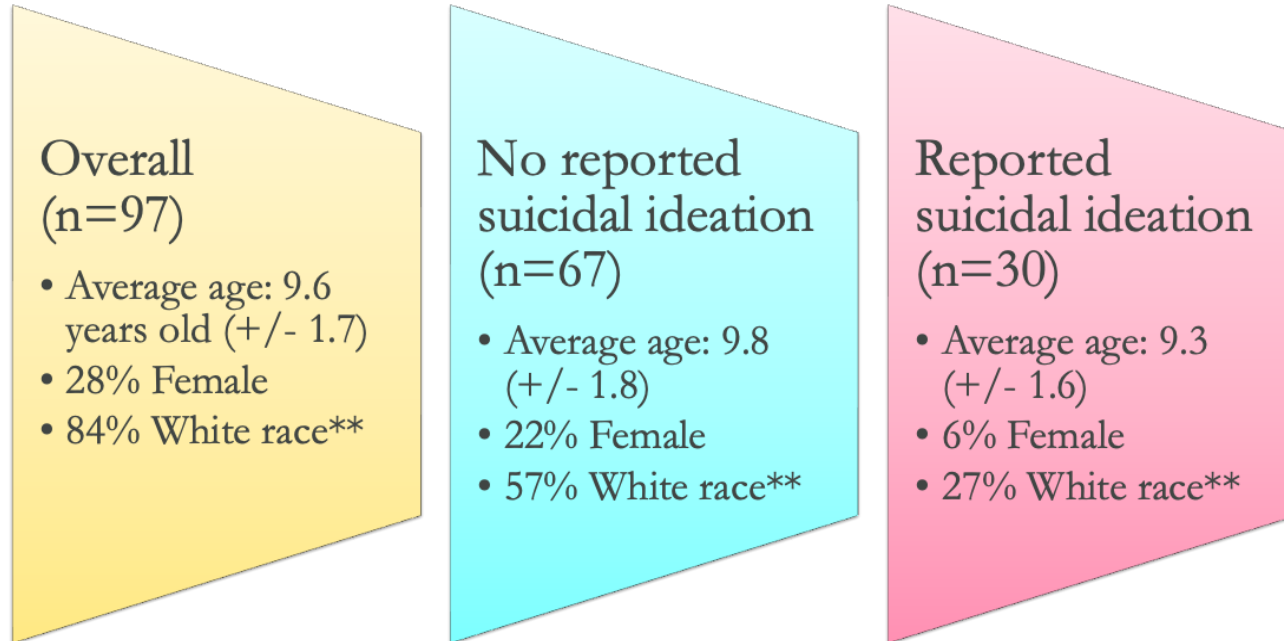
## Methods:

- ❖ 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-1 $\alpha$	11.3	11.3	0.91
MIP-1 $\beta$	17.3	18.9	0.08
MDC	701.9	694.1	0.95
TNF- $\alpha$	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-1 $\beta$ ) 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

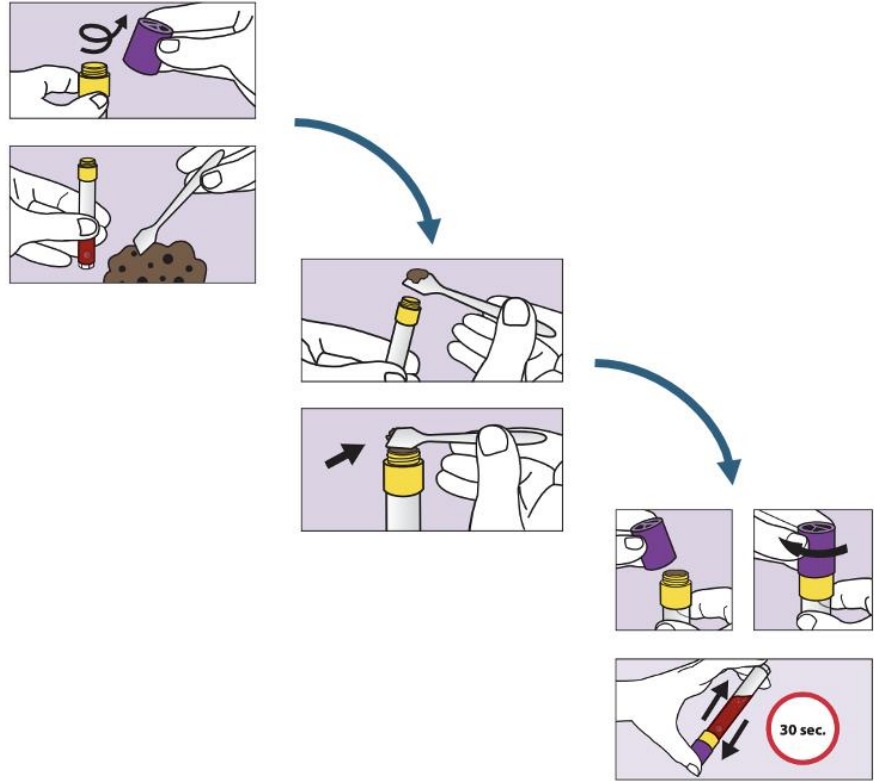
# Sample Collection

- ▶ 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  $-80^{\circ}\text{C}$  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)

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graph TD; A[Sub-cohort analysis of fecal samples (n=50)] --> B[Samples sent to Pacific Northwest National Laboratories for DNA extraction 16s rRNA gene sequencing and raw data processing.]; B --> C[DNA extraction performed]; C --> D[Sequencing performed on an Illumina MiSeq.];
```

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

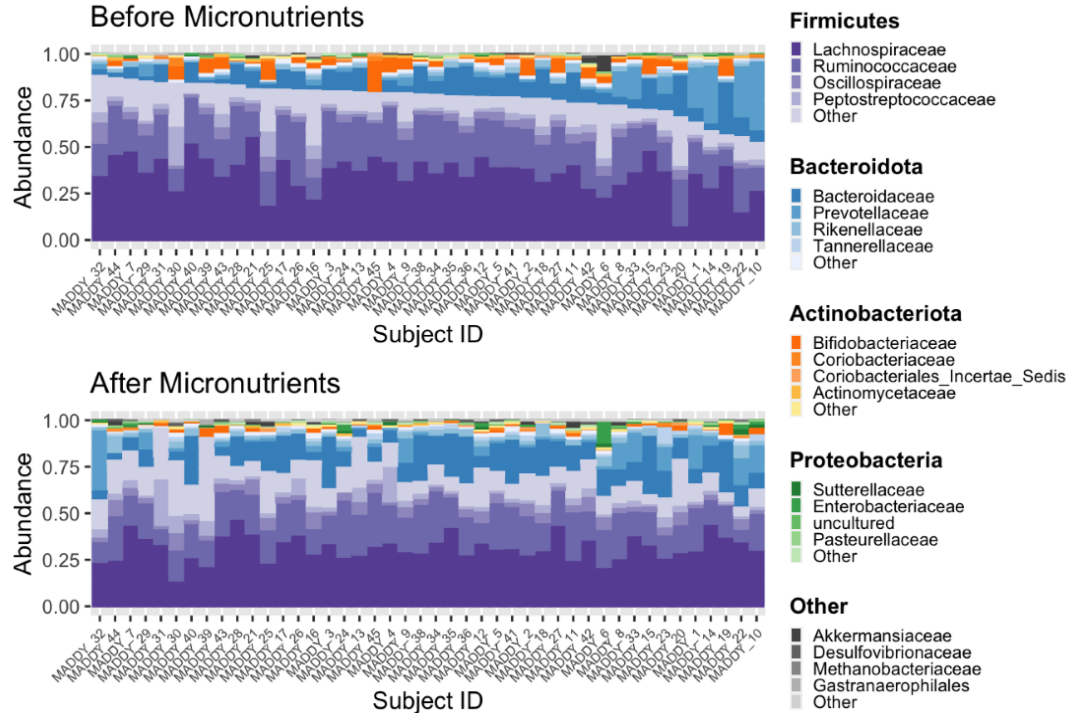
DNA extraction performed

Sequencing performed on an Illumina MiSeq.



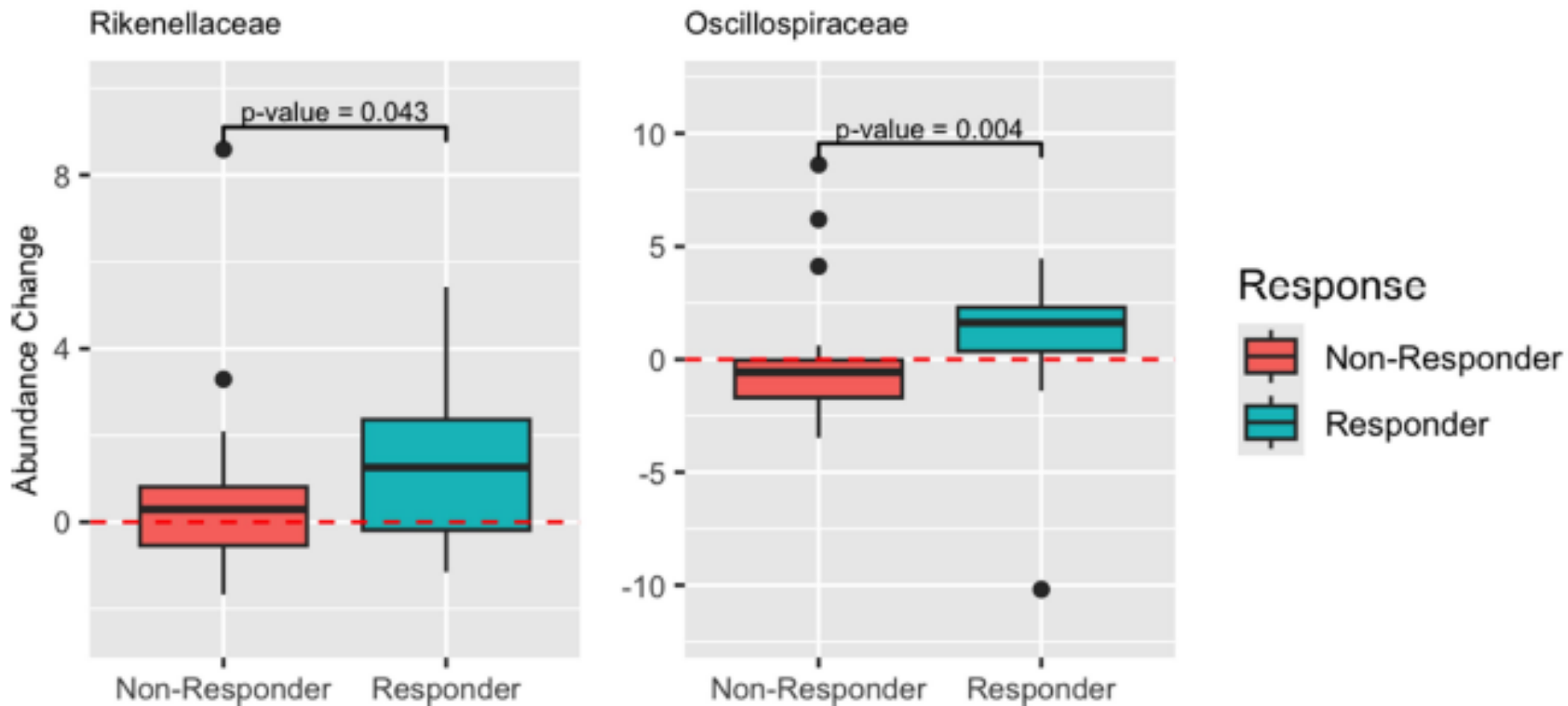
# Microbiome Results

Taxonomic Composition of Samples for Each Subject Before and After Micronutrients Supplementation



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

## Change of Relative Abundance During Micronutrient Intervention



# 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.

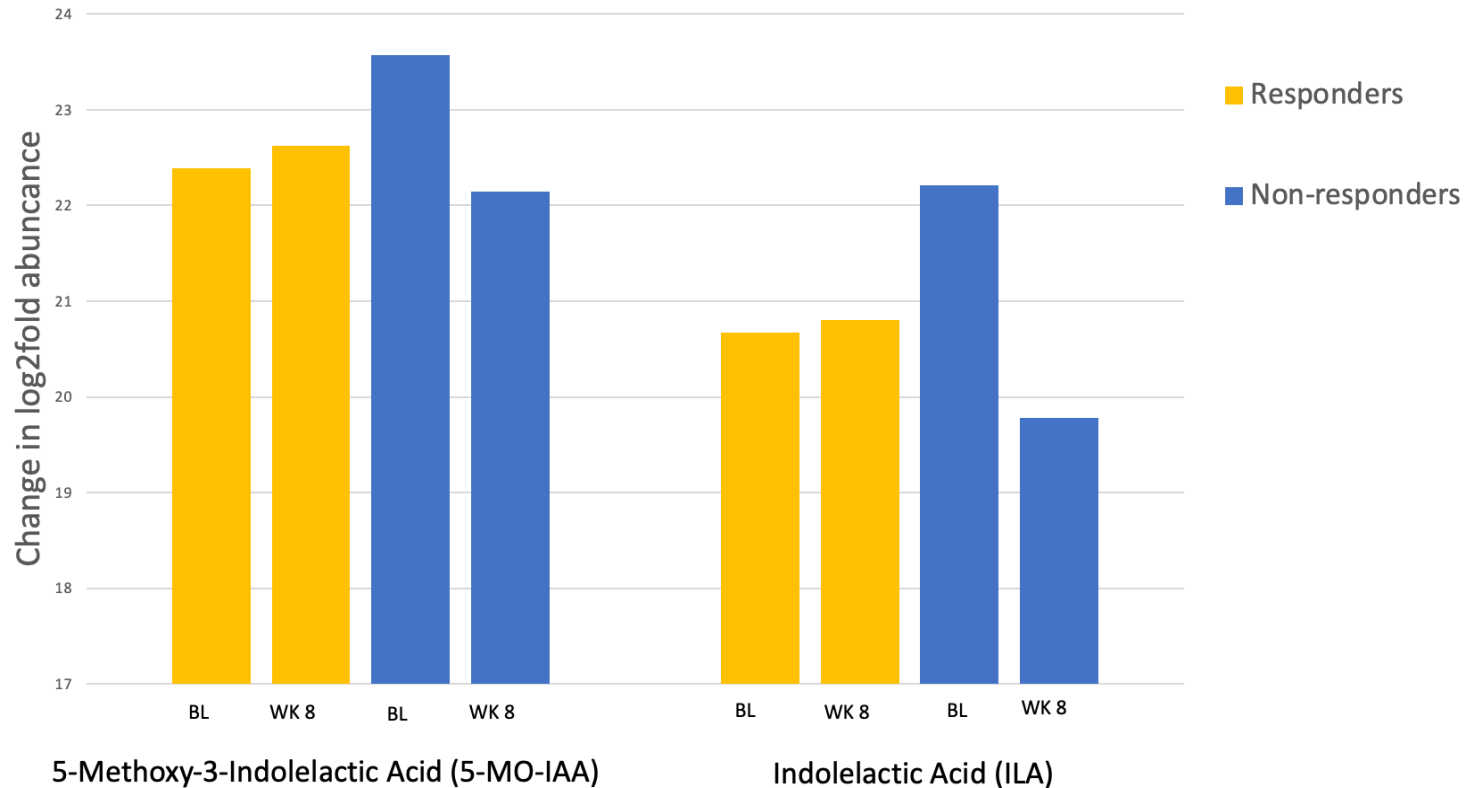
# Metabolite Analysis

**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



# Liquid Urine

- ▷ Collection
  - Pee in a cup
  - Aliquoting samples
  - Freezing ( $-80^{\circ}\text{C}$ ) and storage
- ▷ Validity
  - Gold Standard



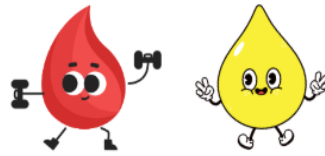


# Dried Urine

- ▶ 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



# Dried Biosampling



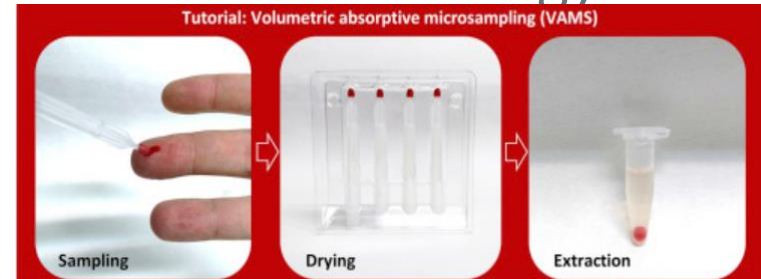
Lamond et al. Journal of Clinical Lab. Analysis 2024  
Protti et al. Analytica Chimica Acta 2019

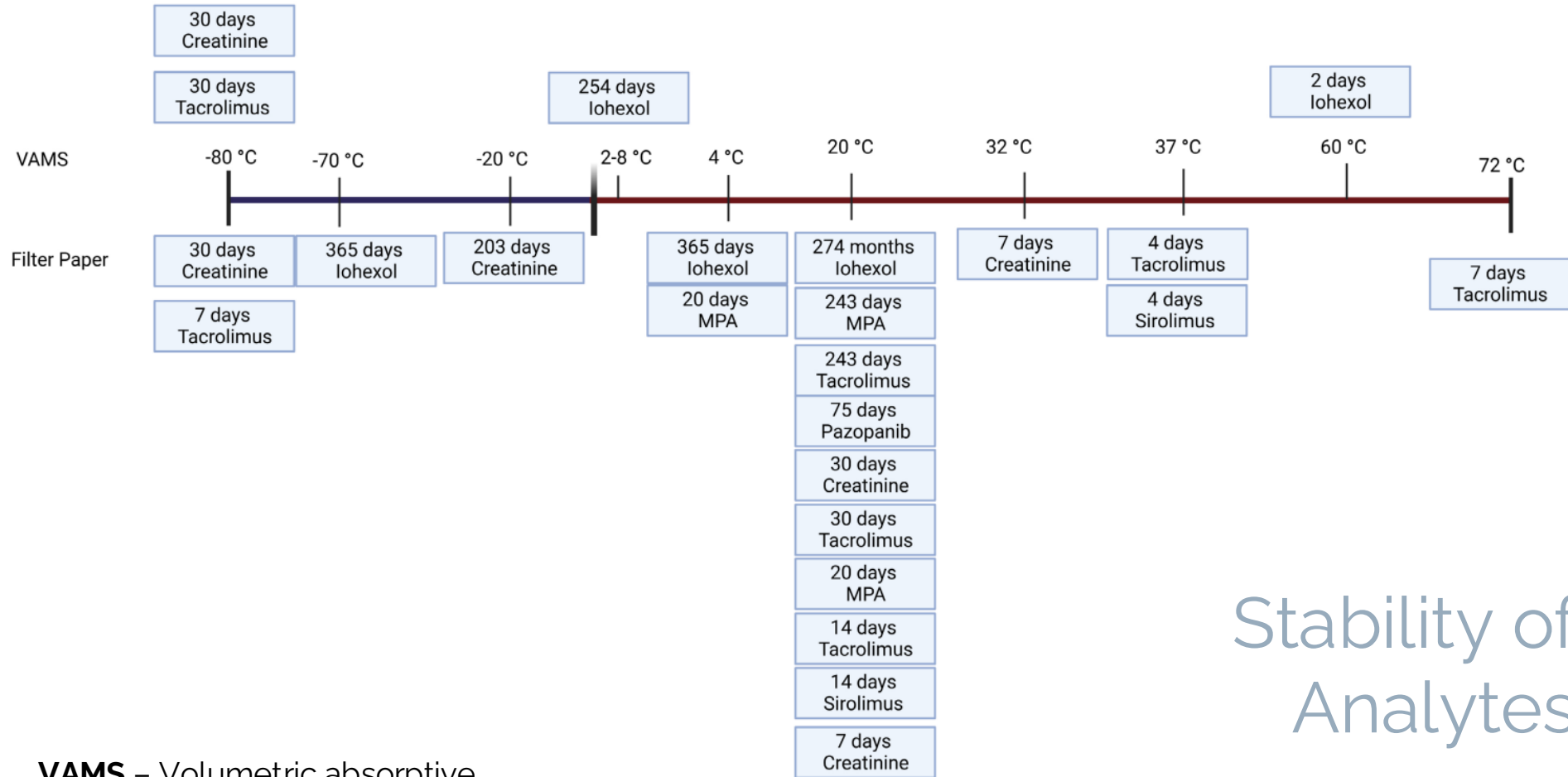
## Pro

- ▷ 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

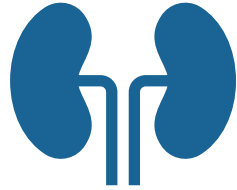
# Glyphosate - MADDY Study

- ▷ 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

# Glyphosate



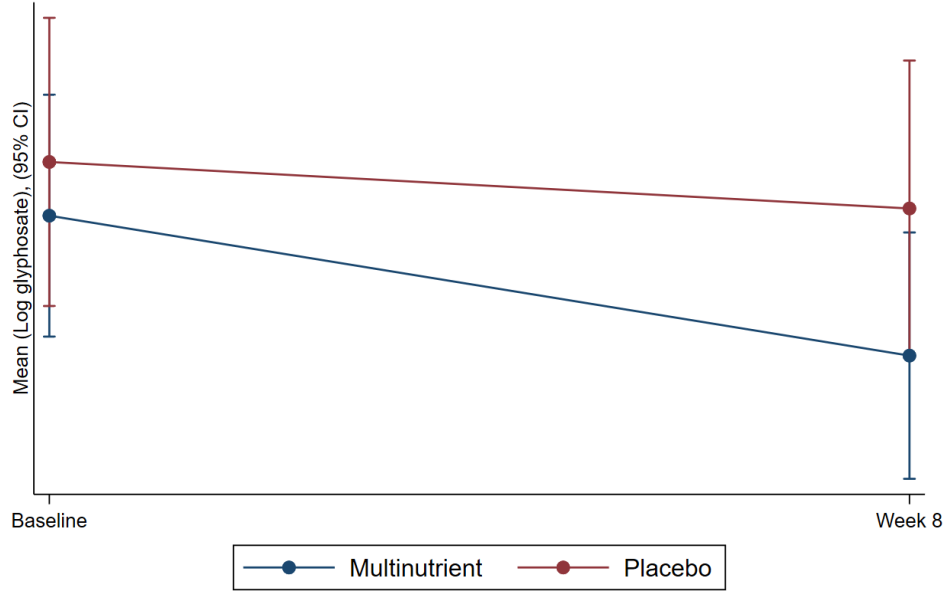
24-hour urine  
collection is best



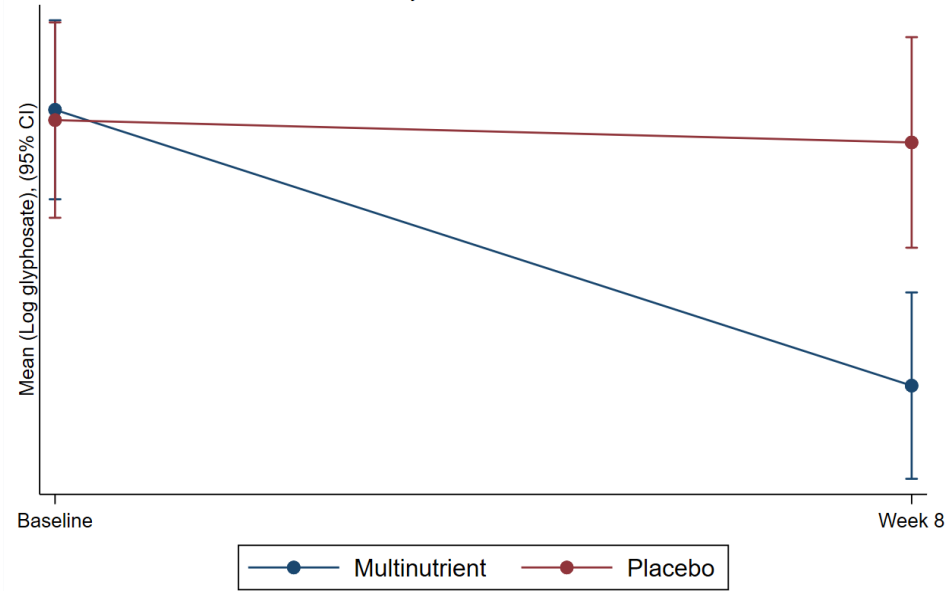
Morning collection  
is second best

# Results

Log of Normalized OHSU glyphosate (ng/mL)  
by treatment & visit



Log of Ohio Normalized glyphosate (ng/mL)  
by treatment & visit



# Liquid Urine



# Dried Urine



OR

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



- Convenient for family and researchers
- Mixed methods/reliability





# Acknowledging our funders

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- **In Canada:** Nutrition and Mental Health Research Fund, administered by the Calgary Foundation

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Health Canada Control #207742  
NCT03252522



FOUNDATION FOR EXCELLENCE  
IN MENTAL HEALTH CARE™



Thank You

SNACK Lab at OHSU

**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



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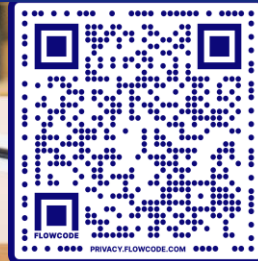
**Ohio State University:** Eugene L. Arnold, MD, Med, Irene Hatsu, PhD, Lisa Robinette, MS; Not Pictured: Chris Zhu, PhD, Shiqi Zhang, PhD, Xinru Pang

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



# Discussion

References:



SNACK Lab:

