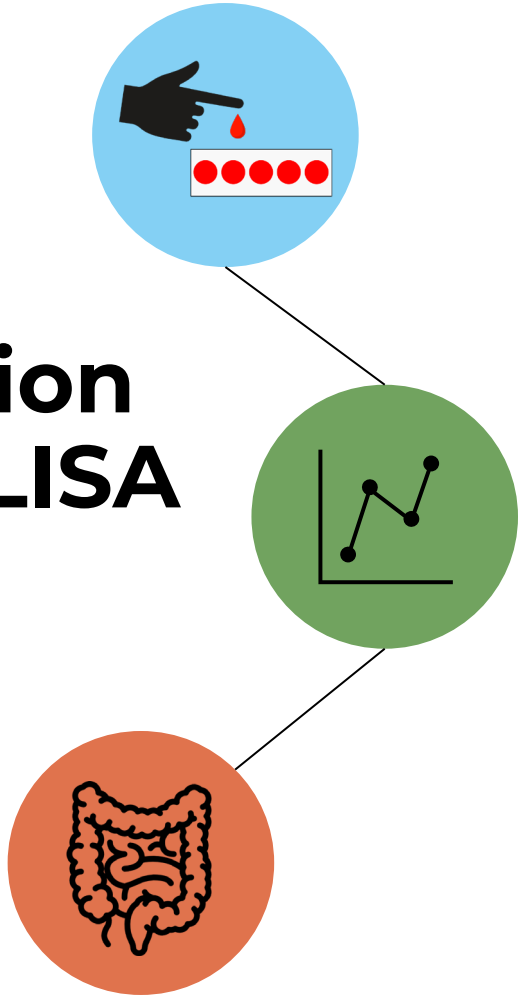
Measurement of intestinal fatty-acid binding protein and intestinal barrier function biomarkers in DBS using ELISA

Elizabeth Kim, M.S.

BAYLOR UNIVERSITY

HEBHL HUMAN EVOLUTIONARY BIOLOGY & HEALTH LAB

NIH National Institutes of Health

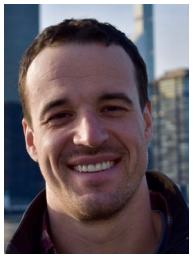


Human Evolutionary Biology and Health Lab

Baylor University

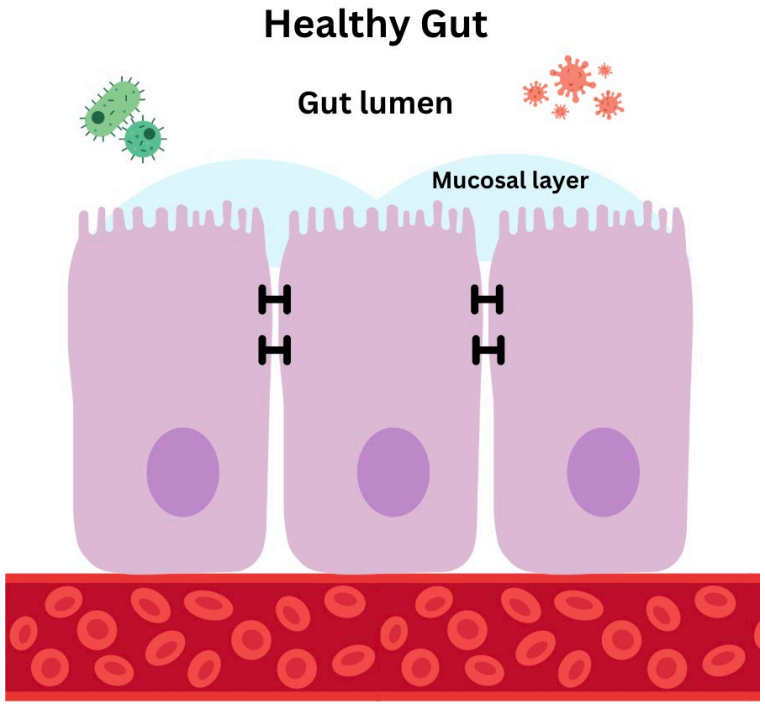
- Investigate bio-energetic pathways shaping variation in human development, metabolism, and health.
- Combine diverse field and laboratory approaches, focusing on participant-friendly measurements and the use of minimally invasive biomarkers.

Dr. Samuel Urlacher (PI)

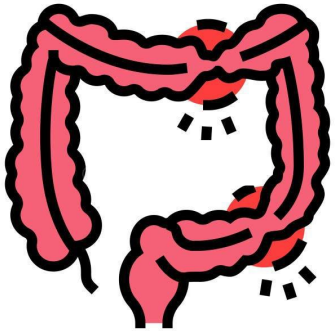


Intestinal function is critical for regulating homeostasis and health

- Nutrient digestion and absorption
- Barrier protection from the external environment
- Immune regulation
- Host-microbe interactions



Intestinal function is critical for regulating homeostasis and health



Irritable bowel syndrome,

Crohn’s disease

Type I diabetes

Non-alcoholic fatty liver disease

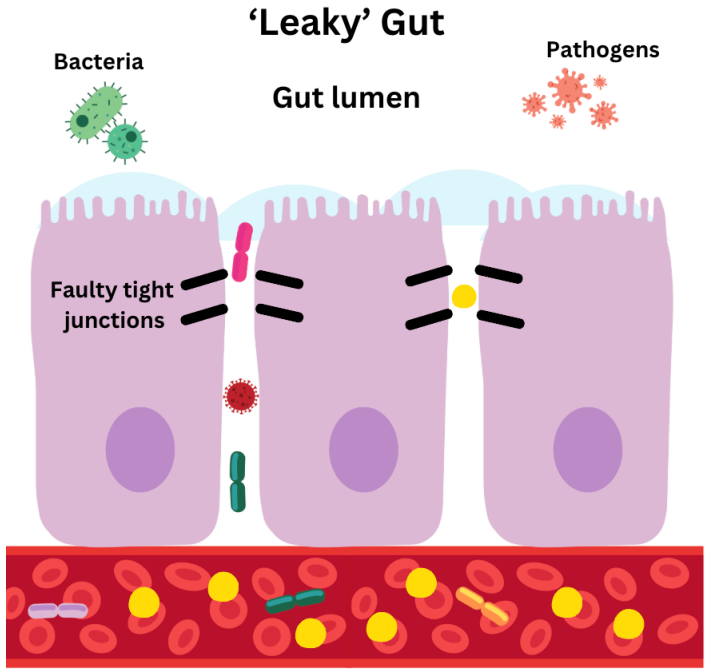
Environmental and lifestyle factors shape variation in intestinal function and health

→ Systemic inflammation

→ Microbial translocation

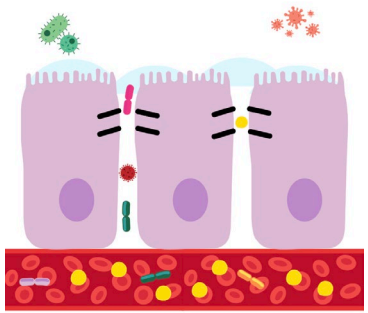
→ Malabsorption

Environmental enteric dysfunction (EED)



Environmental Enteric Dysfunction (EED)

- Acquired subclinical condition of small intestine
- Pathogen exposure from unsanitary living conditions
- Nearly ubiquitous in low-resource and disadvantaged contexts



↑ Gut permeability,
Microbial translocation,
Malabsorption

↑ Immune activity,
Systemic inflammation,
Related energy costs

↓ Growth,
Vaccine efficacy,
Neurodevelopment

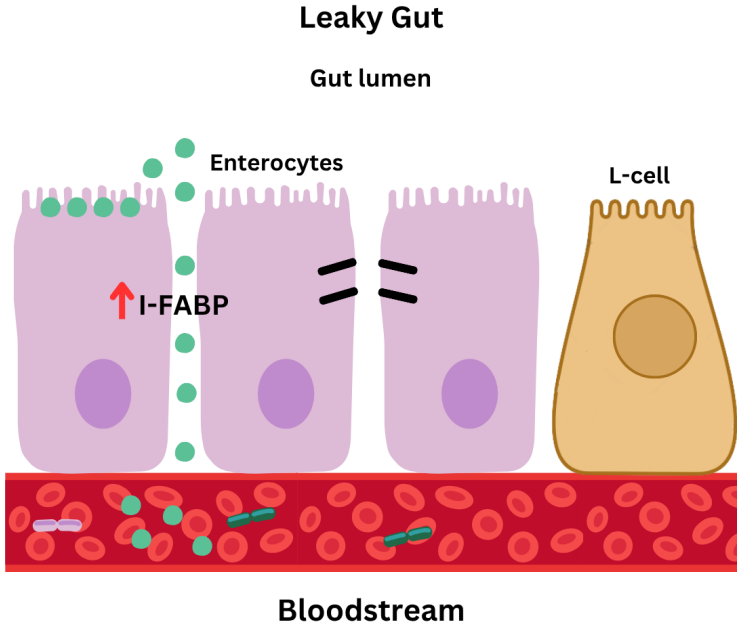
Standardized and objective measures for EED assessment are limited and rely on invasive sampling methods

Objective: validate EED biomarkers in finger-prick dried blood spots (DBS) via ELISA

National Institutes of Health
NIH-NICHD #R15HD106177

<u>Objective</u>: validate EED biomarkers in finger-prick dried blood spots (DBS) via ELISA

1) **Intestinal fatty-acid binding protein (I-FABP)**
   - Cytosolic protein, transports long-chain fatty acids
   - ↑ circulating I-FABP → ↑ intestinal barrier damage

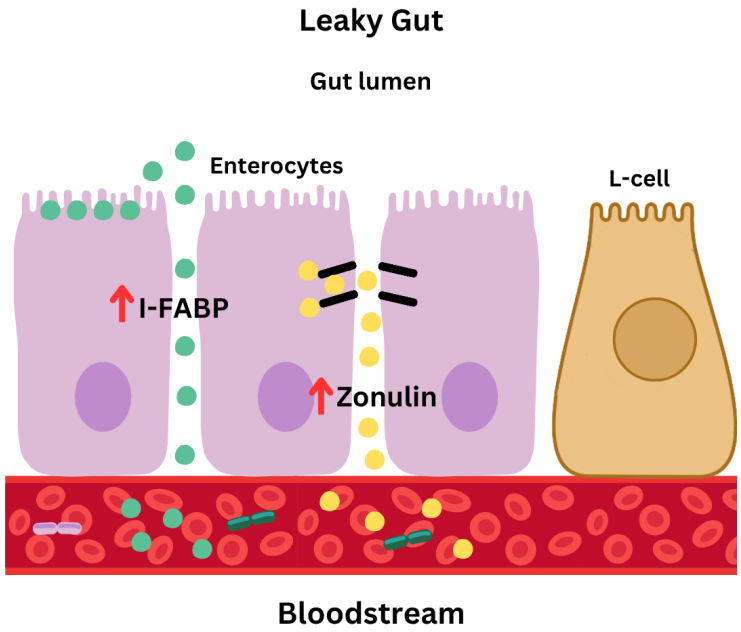


NIH National Institutes of Health
NIH-NICHD #R15HD106177

<u>Objective</u>: validate EED biomarkers in finger-prick dried blood spots (DBS) via ELISA

1) **Intestinal fatty-acid binding protein (I-FABP)**
   - Cytosolic protein, transports long-chain fatty acids
   - ↑ circulating I-FABP → ↑ intestinal barrier damage

2) **Zonulin**
   - Regulator of tight junctions
   - ↑ circulating zonulin → ↑ intestinal permeability



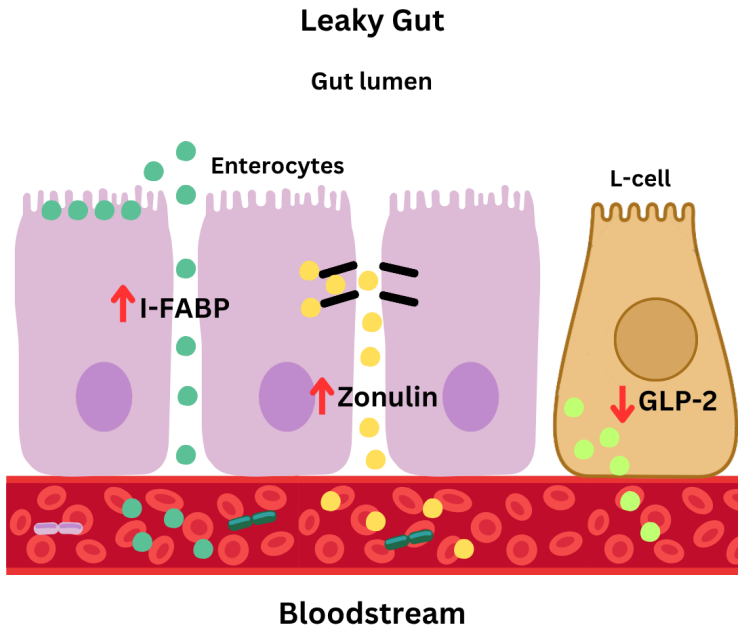
NIH National Institutes of Health
NIH-NICHD #R15HD106177

<u>Objective</u>: validate EED biomarkers in finger-prick dried blood spots (DBS) via ELISA

1) **Intestinal fatty-acid binding protein (I-FABP)**
   - Cytosolic protein, transports long-chain fatty acids
   - ↑ circulating I-FABP → ↑ intestinal barrier damage

2) **Zonulin**
   - Regulator of tight junctions
   - ↑ circulating zonulin → ↑ intestinal permeability

3) **Glucagon-like peptide 2 (GLP-2)**
   - Repair mucosa and promote nutrient absorption
   - ↓ circulating GLP-2 → ↑ intestinal barrier damage, ↓ nutrient absorption



NIH National Institutes of Health
NIH-NICHD #R15HD106177

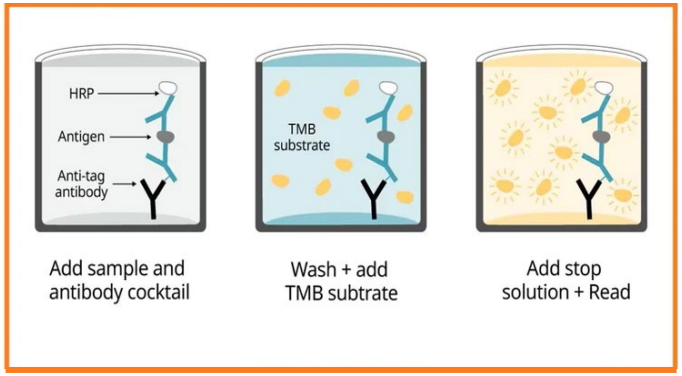
Samples: ‘Waco100’ matched DBS and serum

- Matched finger-prick DBS and serum samples
- Convenience sample of **117 adults** residing in McLennan County, Waco, Texas
- Standard collection procedures
    - Protein saver filter paper cards (Whatman #903)
    - Stored at -80°C until analysis
- Sample collection as part of the Waco COVID Survery

Muehlenbein et al., 2023

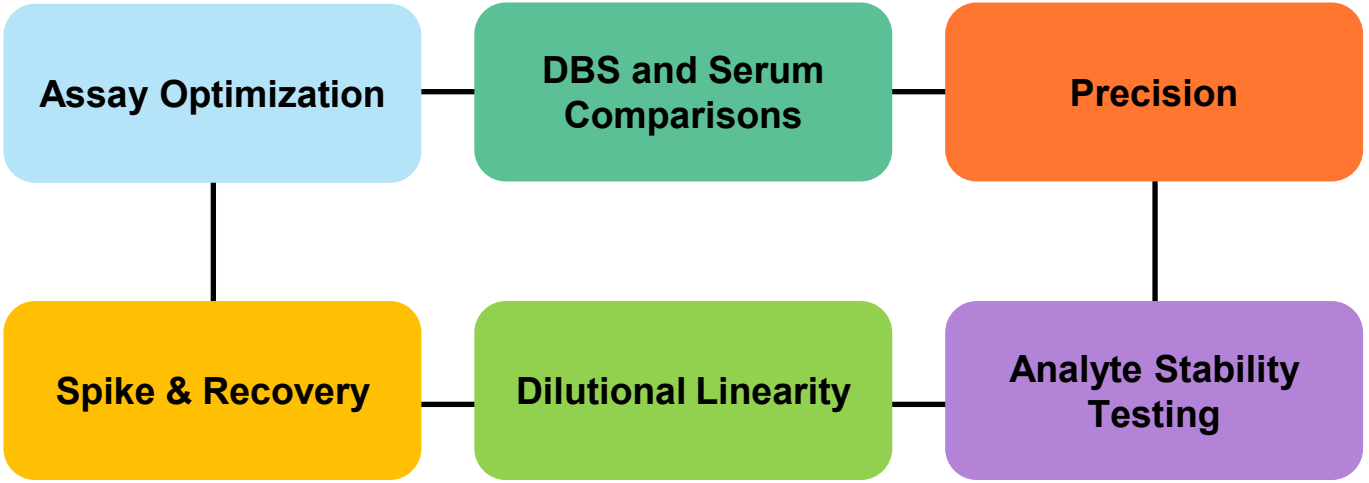
Kit selection: Abcam Human FABP2 ELISA (ab234566)

- Abcam Human FABP2 ELISA Kit (ab234566)
    - Quantitative calorimetric sandwich ELISA
    - Validated for urine, serum, plasma, and cell culture samples
- Assay range: 12.5 pg/mL to 800 pg/mL
- Low sensitivity: 1.7 pg/mL
- Relatively minimal sample requirement (50uL/well)
- Short total incubation time: 70 minutes



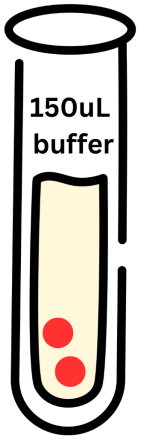
Validation Testing and Methods

- Following established protocols developed by McDade



Assay Optimization

- Determine ideal DBS sample elution parameters
- DBS elution strategy: **2 small (3.2mm^2) punches eluted in 150uL** of Sample Diluent NS
    - Eluted overnight for 12 hours at 4^{0}C
- Serum samples diluted 1:19 with Sample Diluent NS

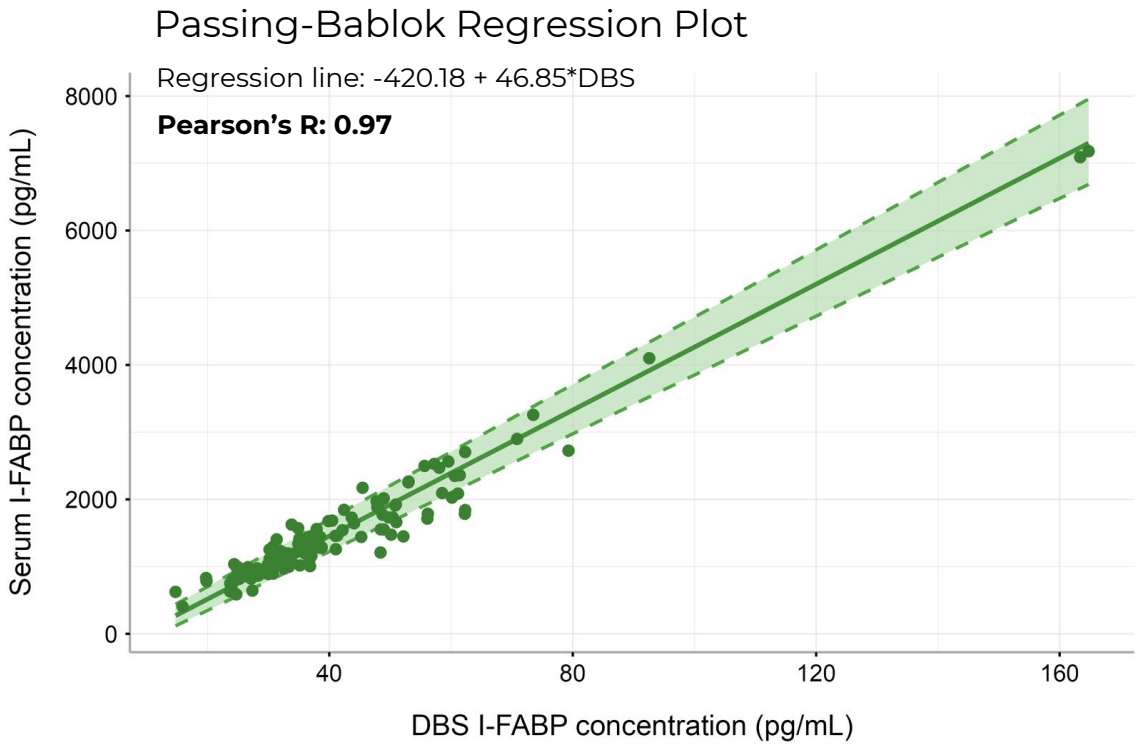


DBS and Serum Comparisons

I-FABP was quantified in all DBS samples (*n* = 117)

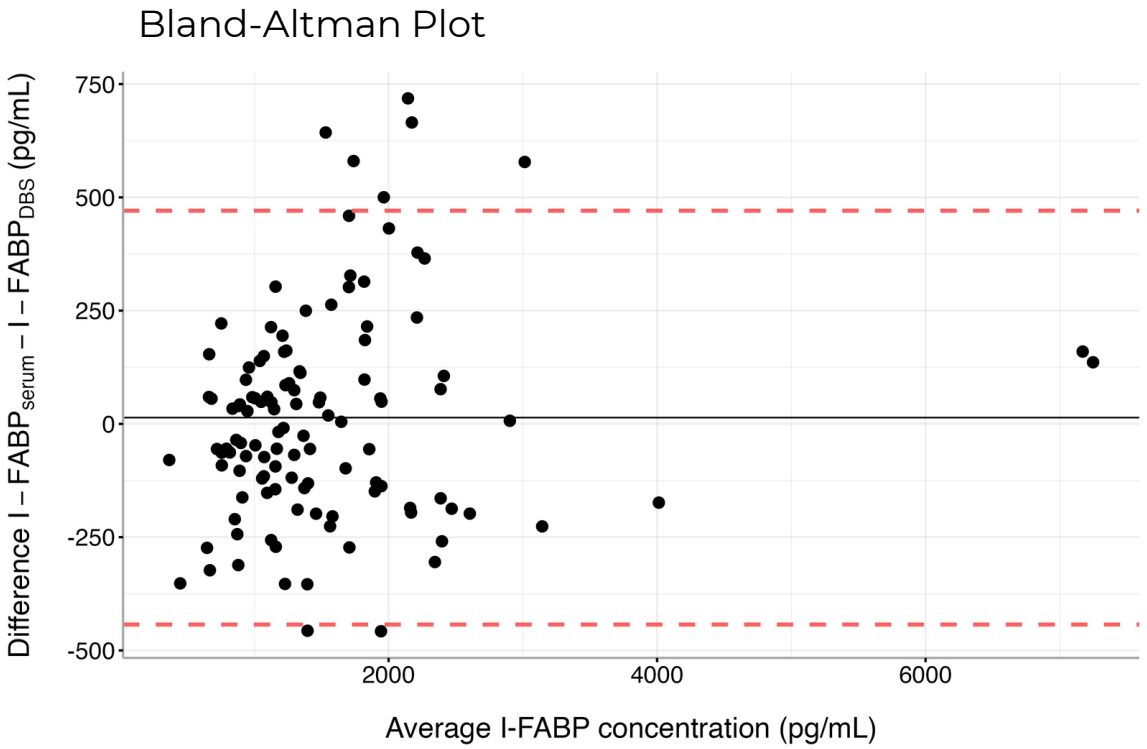
Mean I-FABP in DBS:
41.97pg/mL ± 21.2 (SD)

Strong linear relationship between DBS and serum I-FABP
(Pearson's R: 0.97)



DBS and Serum Comparisons

Minimal bias observed between serum I-FABP and DBS I-FABP levels **(bias = 13.9)**



Precision

Intra-assay CV

Average CV for duplicate measures of DBS samples

4.0 %

Inter-assay CV

Average CV for DBS control included on each plate

3.9 %

Limit of Detection

Calculated as the concentration of the mean OD value for all blank wells + 2 SDs

5.27pg/mL

Spike and Recovery

Average % recovery: 101.8%

Low Spike	Med Spike	High Spike
+208 pg/mL I-FABP	+385 pg/mL I-FABP	+536 pg/mL I-FABP
101.9% recovery	100.5% recovery	102.8% recovery

Dilutional Linearity

Average % recovery: 111.2%

1:2 Dilution

106.8%
recovery

1:4 Dilution

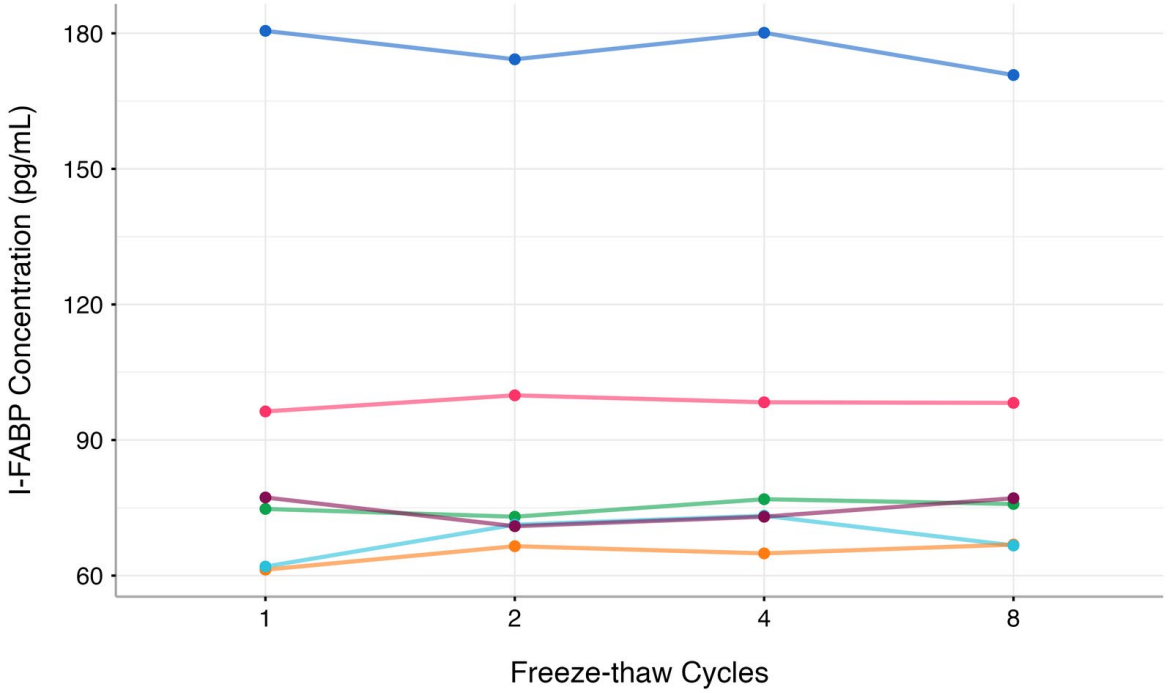
118%
recovery

1:8 Dilution

108.9%
recovery

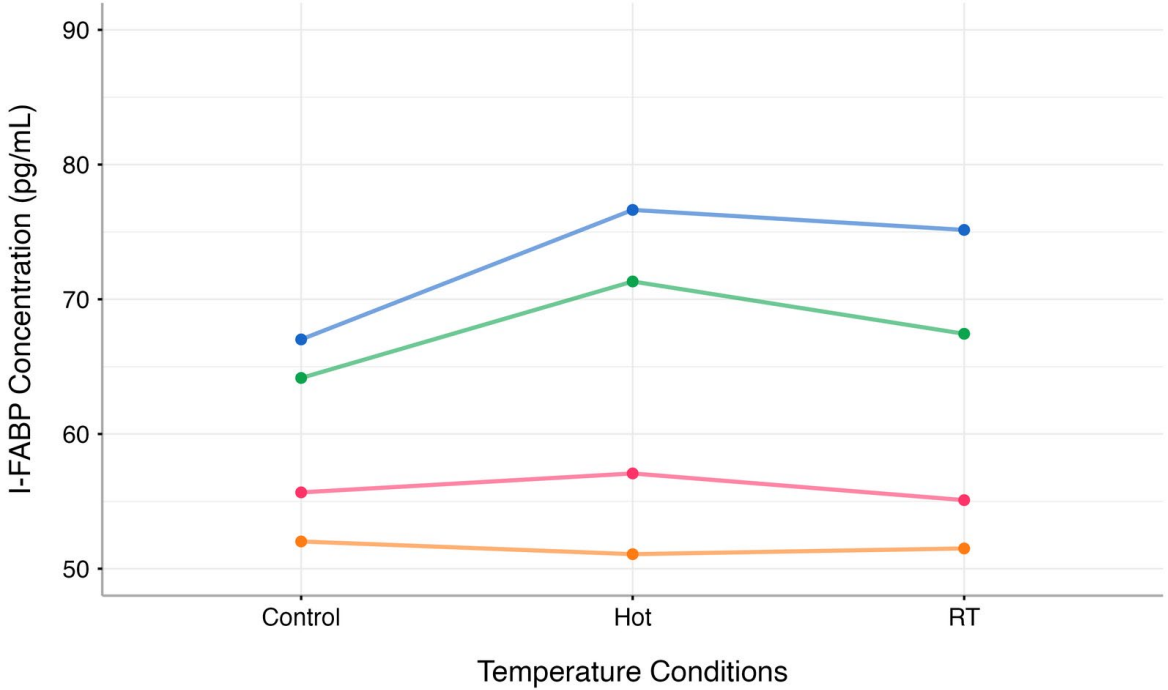
Analyte Stability

No significant differences in I-FABP concentrations across 1, 2, 4, and 8 F/T cycles ($p > 0.05$)



Analyte Stability

No significant differences in I-FABP concentrations across control, hot, and RT conditions ($p > 0.05$) for at least 72 hours



I-FABP can be reliably quantified in DBS using ELISA

Abcam Human FABP2 ELISA Kit (ab234566)

Assay Optimization

DBS elution:
2 small (3.2mm^2) punches in 150uL diluent

DBS and Serum Comparisons

Strong linear relationship **(Pearson's R: 0.97)**

No obvious bias (bias = 13.9)

Precision

Intra-assay CV: **4.0%**

Inter-assay CV: **3.9%**

LOD: **5.27pg/mL**

Spike and Recovery

Average percent recovery:

101.8%

Dilutional Linearity

Average percent recovery:

111.2%

Analyte Stability

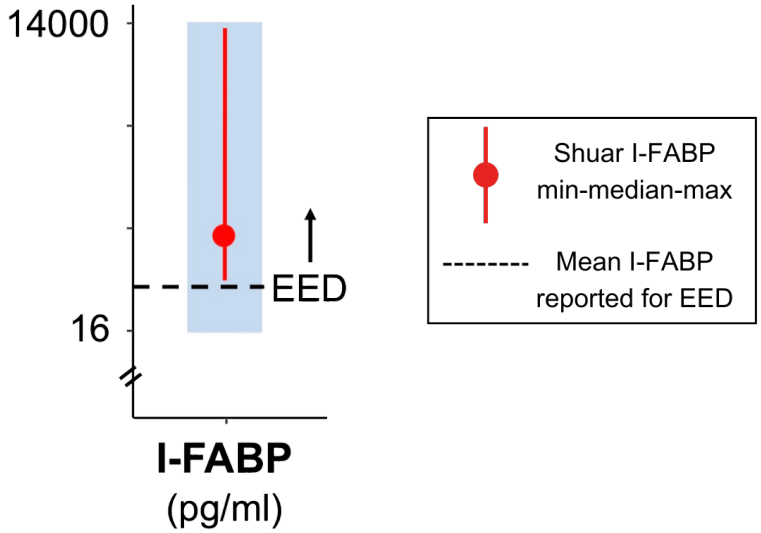
I-FABP **stable** for up to **8 F/T cycles** and at least **72 hours** at **RT** and **hot** conditions ($p > 0.05$).

DBS I-FABP measurement in Amazonian Shuar Children

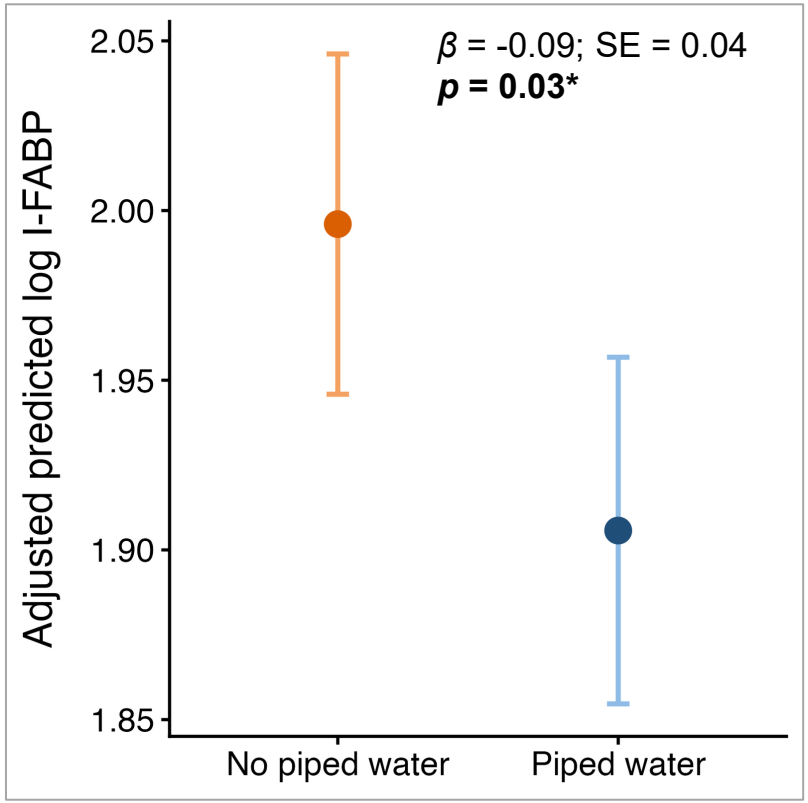
- Indigenous population living in Amazonian Ecuador
- Experience varying rates of market integration
- High immune activity and pathogen burden
- Intestinal inflammation and parasites
- 41% of Shuar children are stunted

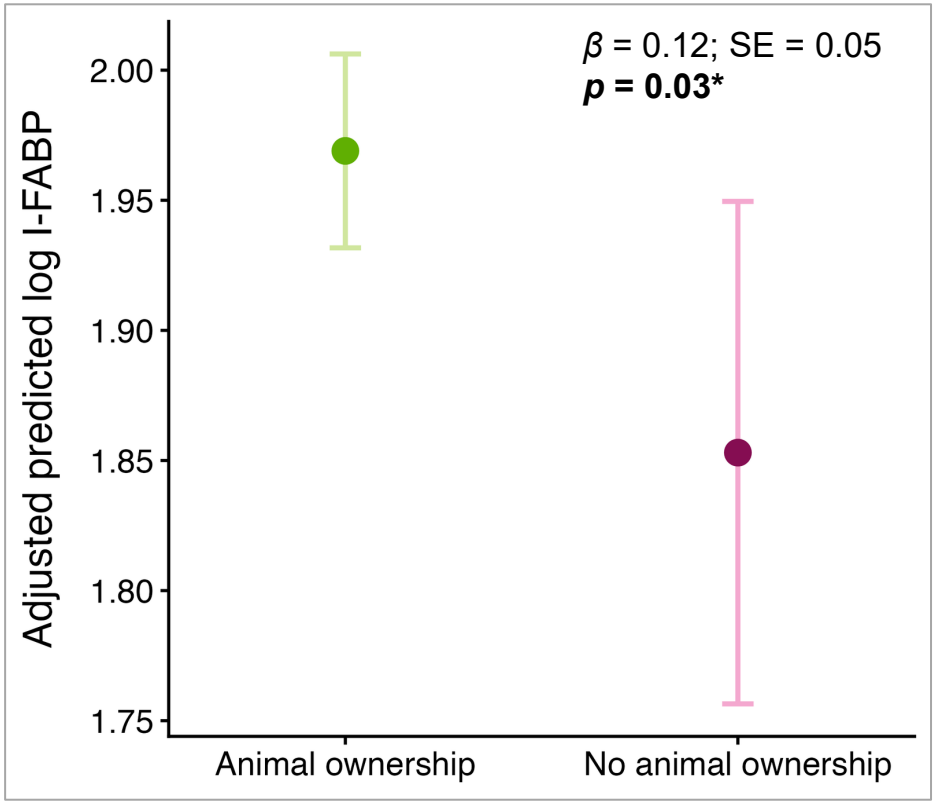
DBS I-FABP measurement in Amazonian Shuar Children

- 312 Shuar children, ages 4 – 12 years old
- Demonstrate **high levels of I-FABP** indicative of **EED**

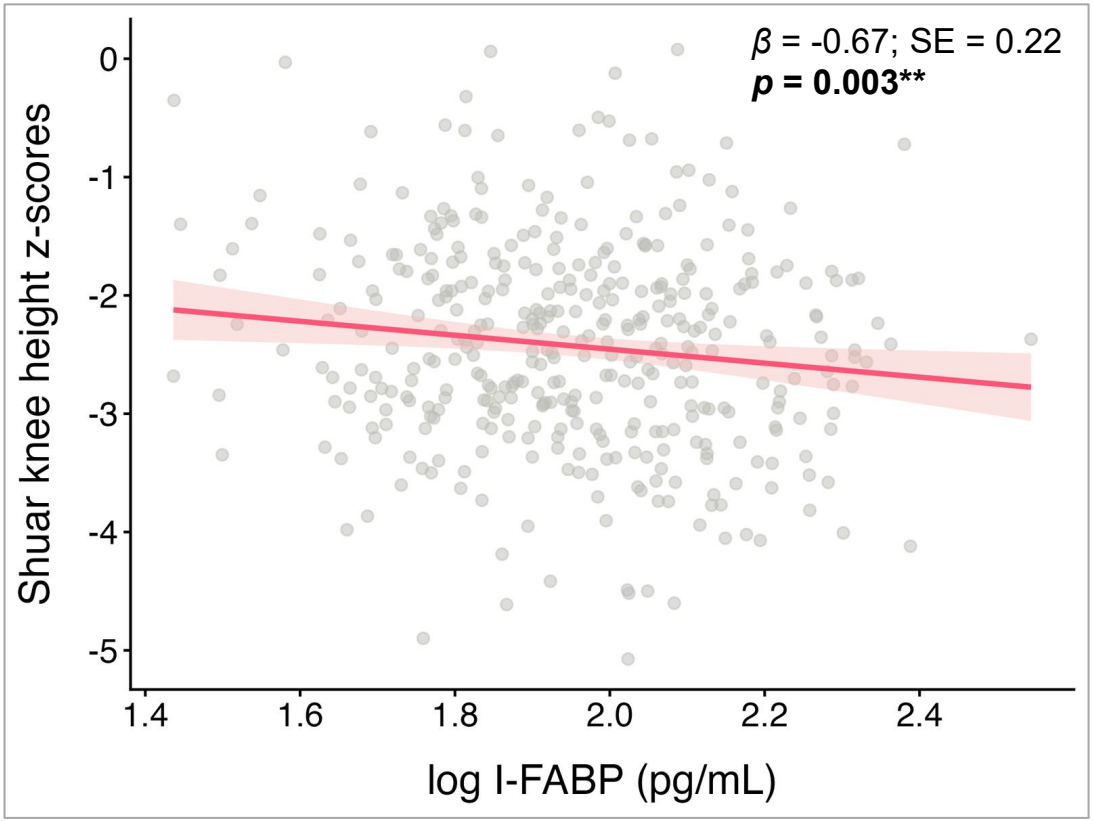


Shuar children's IFABP levels are significantly associated with household living conditions



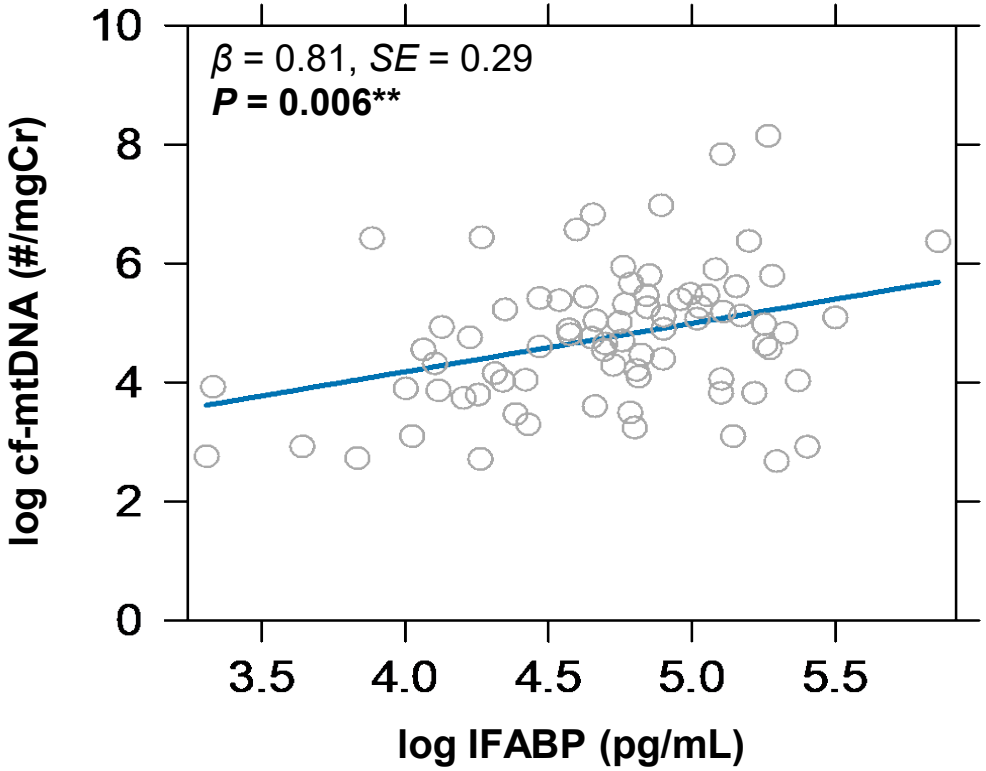


Shuar children's IFABP levels are significantly associated with growth measures



**Trending significant
(p =0.05) with Shuar HAZ**

Shuar children's IFABP levels are significantly associated with cell-free mitochondrial DNA



Thank you!

Questions?

Elizabeth Kim, M.S.

Email: lizzy_kim1@baylor.edu

HEBHL Team

We thank the Waco COVID participants and Shuar families for their participation.

BAYLOR UNIVERSITY 1845

HEBHL HUMAN EVOLUTIONARY BIOLOGY & HEALTH LAB

NIH National Institutes of Health

WACO FAMILY MEDICINE

SHUAR HEALTH & LIFE HISTORY PROJECT