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The U.S. Government's Global Hunger & Food Security Initiative

# *Assessing maternal environmental enteric dysfunction and its association with adverse birth outcomes in Uganda*

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# Disclosures

I have no disclosures in relation to this presentation.

# Presentation Outline

- Introduction
  - Clinical presentation
  - Assessment
  - Mechanisms (EED and poor growth)
- Maternal EED Study in Mukono, Uganda
  - Background
  - Methods
  - Results
- Next Steps

# Environmental enteric dysfunction

- EED is characterized by changes in the structure and function of the small intestine:
  - Blunting of the villi
  - Reduced epithelial surface area and absorptive capacity
  - Altered mucosal barrier integrity
  - Intestinal and systemic Inflammation

- It is postulated that EED develops throughout infancy as the result of chronic exposure to enteropathogens due to living in poor water, sanitation, and hygiene (WASH) conditions.

Despite being considered the "gold standard" for diagnosing EED, the invasive nature of intestinal biopsy makes it ill-suited for the evaluation of EED in field settings.

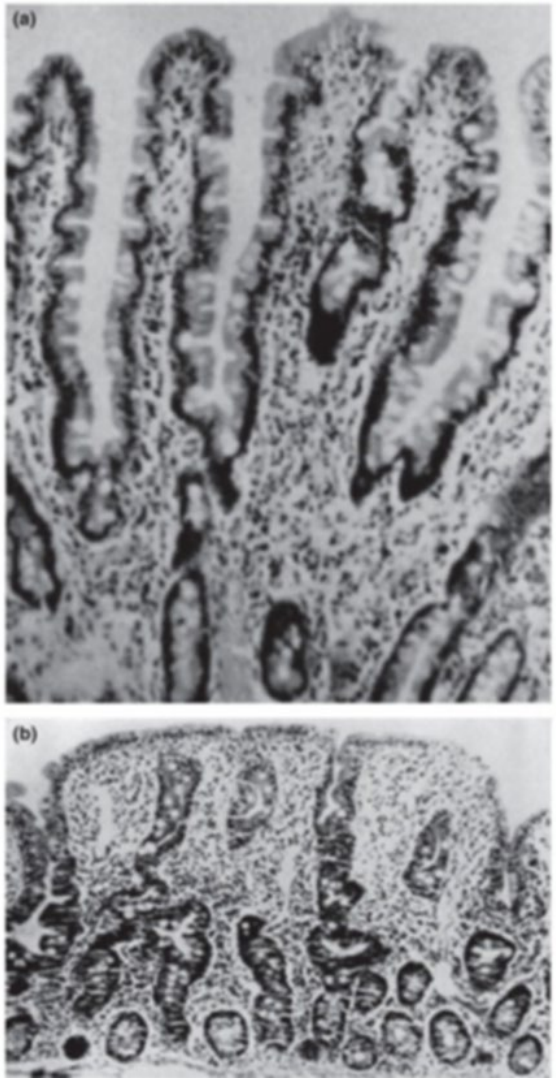
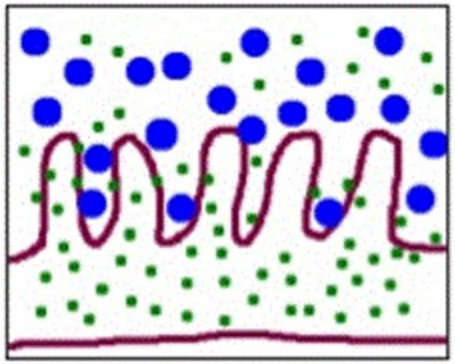



*Figure: Jejunal biopsies of normal (a) and diseased (b) intestines*

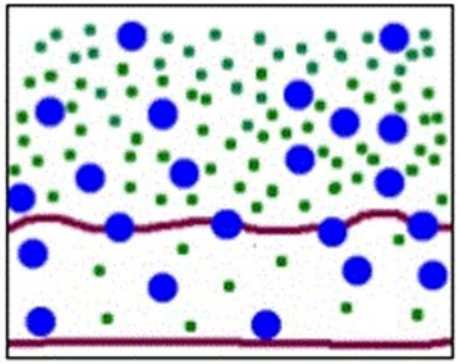
# L:M Test

- The urinary lactulose: mannitol (L:M) dual sugar test is the most commonly used proxy test.
  - Participant drinks a solution containing the two sugars
  - All urine is collected for the duration of the test (4-5 hours)
  - Lactulose = disaccharide, a large sugar that is minimally absorbed in the small intestine, except in the case of intestinal permeability
    - EED = ↑ lactulose absorption
  - Mannitol = monosaccharide, a smaller sugar, which is absorbed proportional to intestinal absorptive capacity
    - EED = ↓ mannitol absorption
  - EED= High L:M ratio

# L:M Test Cont.



Healthy Intestine = Low L:M ratio



EE = High L:M ratio

Courtesy of Audrie Lin and Steve Luby
http://www.slideshare.net/aashishysg/audrie-2013-0731-delhi-conference-ee-pilot-final

Lactulose sugar

Mannitol sugar

# L:M Test Limitations

- Time-consuming (5+ hours)
- Burdensome
- Expensive
- High rate of test failure
  - E.g. Spilled/leaked urine or stool contamination
- Lacks formal evaluation studies
- Measures absorptive capacity and permeability, but not necessarily other domains of EED
- Is inconsistently correlated with EED symptoms and growth outcomes in young children

**Table 1. Biomarkers to Assess Environmental Enteric Dysfunction**

| Category | Biomarkers |
|---|---|
| 1. Intestinal absorption and mucosal permeability | D-xylose, mannitol, or rhamnose absorption; lactulose paracellular uptake; α1-anti-trypsin leakage into gut lumen |
| 2. Enterocyte mass and function | Plasma citrulline and/or conversion of alanyl-glutamine to citrulline, lactose tolerance test (as a marker of brush border damage) |
| 3. Inflammation | Plasma cytokines, stool calprotectin, myeloperoxidase, or lactoferrin |
| 4. Microbial translocation and immune activation | Stool neopterin, plasma LPS core antibody and/or LPS binding protein, circulating soluble CD14 |

Abbreviation: LPS, lipopolysaccharide.

Keusch et al. CID 2014: 59 (Suppl 4) S207

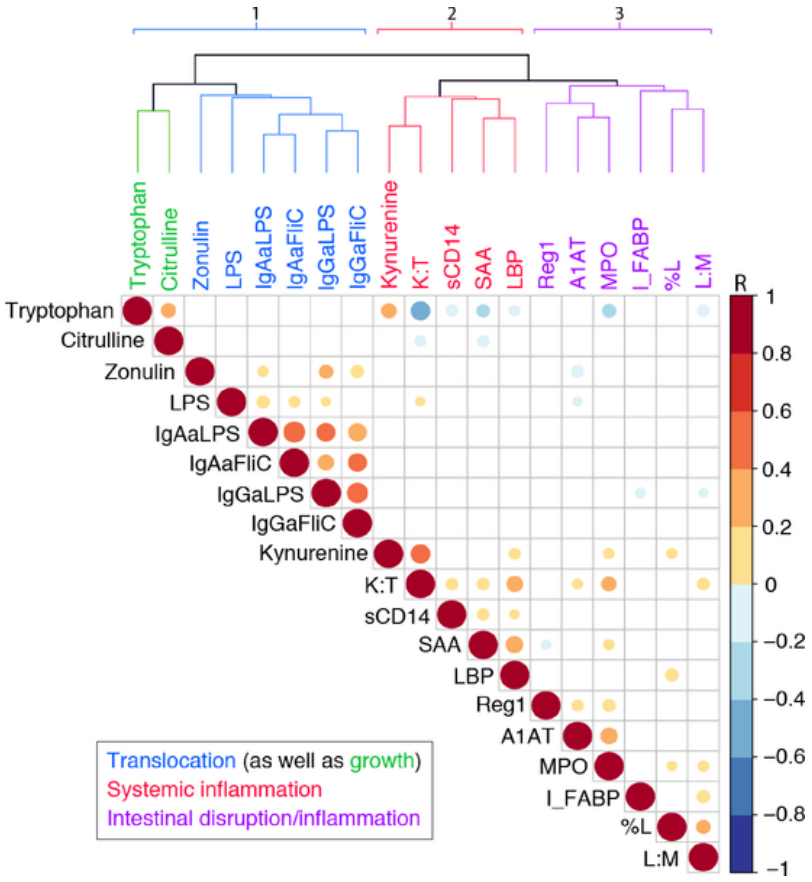



**Figure: Cluster dendrogram with Pearson correlations among those biomarkers with ≥274 values**

Guerrant RL, Leite AM, Pinkerton R, Medeiros PHQS, Cavalcante PA, et al. (2016) Biomarkers of Environmental Enteropathy, Inflammation, Stunting, and Impaired Growth in Children in Northeast Brazil. PLOS ONE 11(9): e0158772. https://doi.org/10.1371/journal.pone.0158772

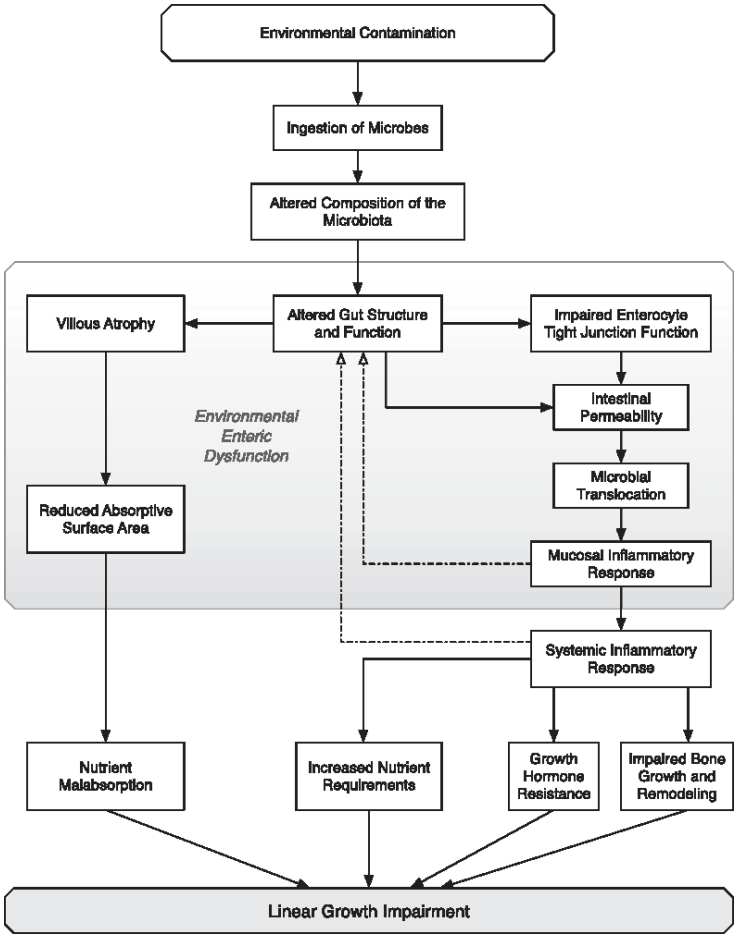
# Anti-Flagellin/Anti-LPS Igs

Flagellin = bacterial protein that mediates bacterial motility

LPS = major structural component of bacteria

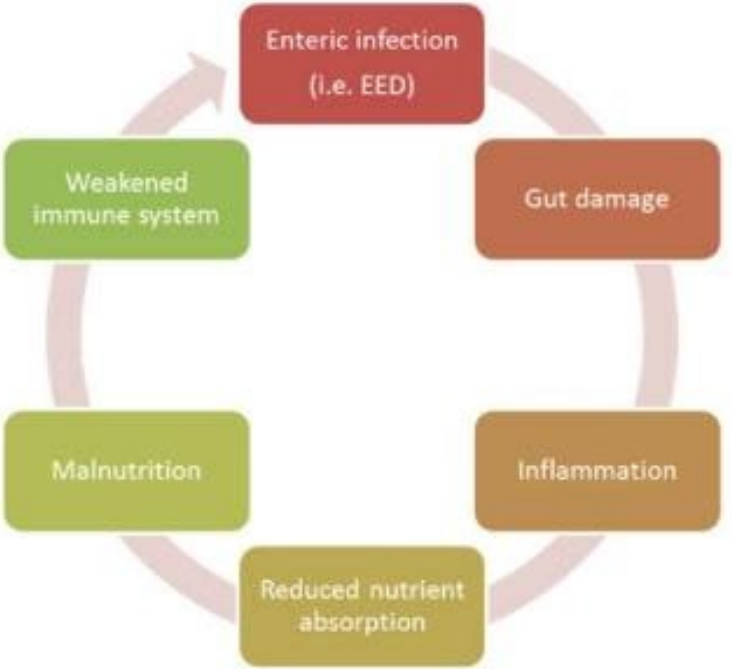
Both are commonly found in the gut lumen

In the case of microbial translocation, anti-flagellin and angi-LPS antibodies are produced and can be identified in serum




Mbuya, Mduduzi NN, and Jean H. Humphrey. "Preventing environmental enteric dysfunction through improved water, sanitation and hygiene: an opportunity for stunting reduction in developing countries." *Maternal & child nutrition* 12 (2016): 106-120.

# EED and Poor Growth




http://www.communityledtotalsanitation.org/resource/environmental-enteric-dysfunction-overview

# Background

- EED has been linked to poor growth outcomes in infants and young children.

- However, the role of maternal EED as a risk factor for adverse birth outcomes is less established.
  - Women with Crohn's disease (CD) / inflammatory bowel disease (IBD) have pregnancies marked by higher rates of preterm birth, small-for-gestational-age (SGA) infants, and other complications

# Objective

To examine the association between EED biomarkers (L:M ratios and anti-flagellin/anti-LPS Igs) in pregnant women aged 18-45 years and subsequent adverse birth outcomes (shorter gestational age, lower birth weight and length, and smaller head circumference) in Mukono District, Uganda

# Study Setting: Mukono, Uganda


# Methodology

- Prospective cohort study (n=258) conducted Feb- and Nov 2017
- Pregnant women were recruited at their first prenatal visit to Mukono Health Center IV (about 16 weeks gestation)
- Associations were assessed with STATA 15 software using (multivariate) linear regression models

| Inclusion criteria | Exclusion criteria |
|---|---|
| Between 18 and 45 years old | Severely malnourished (BMI < 16.0 kg/m2) |
| Residing within 10 km. of Mukono Town | Severely anemic (Hb < 7 g/dl) |
| Carrying a singleton pregnancy | HIV-positive |
| | Planning to move from Mukono District |

# Visit Schedule

| Visit | Time | Location | Description |
|---|---|---|---|
| #1: Enrollment visit (n=254) | After first prenatal visit (9-27 weeks gestation) | MHC IV | • Ultrasound scan<br>• Hb test/blood pressure tests<br>• Venous blood draw<br>• Anthropometry (height, weight, MUAC)<br>• Questionnaire |
| #2: L:M test (n=247) | < 1 week after enrollment visit | Participants' residence | • Solution containing 5 grams of lactulose and 2 grams of mannitol<br>• 4-hour timed urine collection |
| #3: Follow-up visit (n=236) | 3 weeks prior to participants' EDD | Participants' residence | • Anthropometry (weight, MUAC)<br>• Questionnaire<br>• Water quality test |
| #4: Delivery visit (n=232 total, 220 born alive) | Within 48 hours of delivery | Participants' residence or health facility | • Infant anthropometry (length, weight, head circumference) |

# Analysis of samples

- Maternal EED was assessed via the L:M test (HPLC, Baylor College of Medicine) and serum concentrations of antibodies to the bacterial components flagellin and LPS (ELISA, Georgia State University)

# Biomarkers of maternal environmental enteric dysfunction are associated with shorter gestation and reduced length in newborn infants in Uganda

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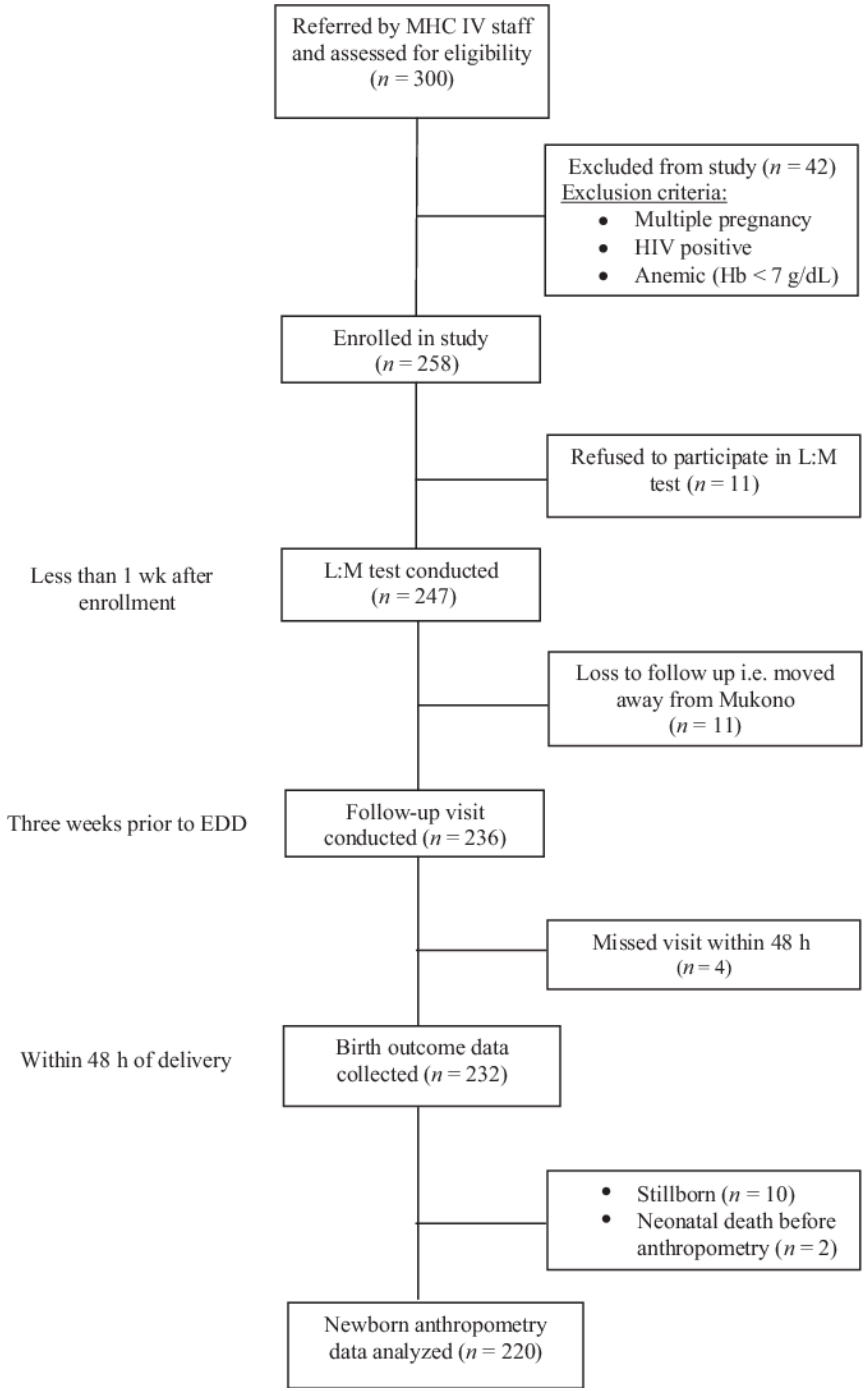

Referred by MHC IV staff and assessed for eligibility (n = 300)
Excluded from study (n = 42)
Exclusion criteria:
Multiple pregnancy
HIV positive
Anemic (Hb < 7 g/dL)
Enrolled in study (n = 258)
Refused to participate in L:M test (n = 11)
Less than 1 wk after enrollment
L:M test conducted (n = 247)
Loss to follow up i.e. moved away from Mukono (n = 11)
Three weeks prior to EDD
Follow-up visit conducted (n = 236)
Missed visit within 48 h (n = 4)
Within 48 h of delivery
Birth outcome data collected (n = 232)
Stillborn (n = 10)
Neonatal death before anthropometry (n = 2)
Newborn anthropometry data analyzed (n = 220)

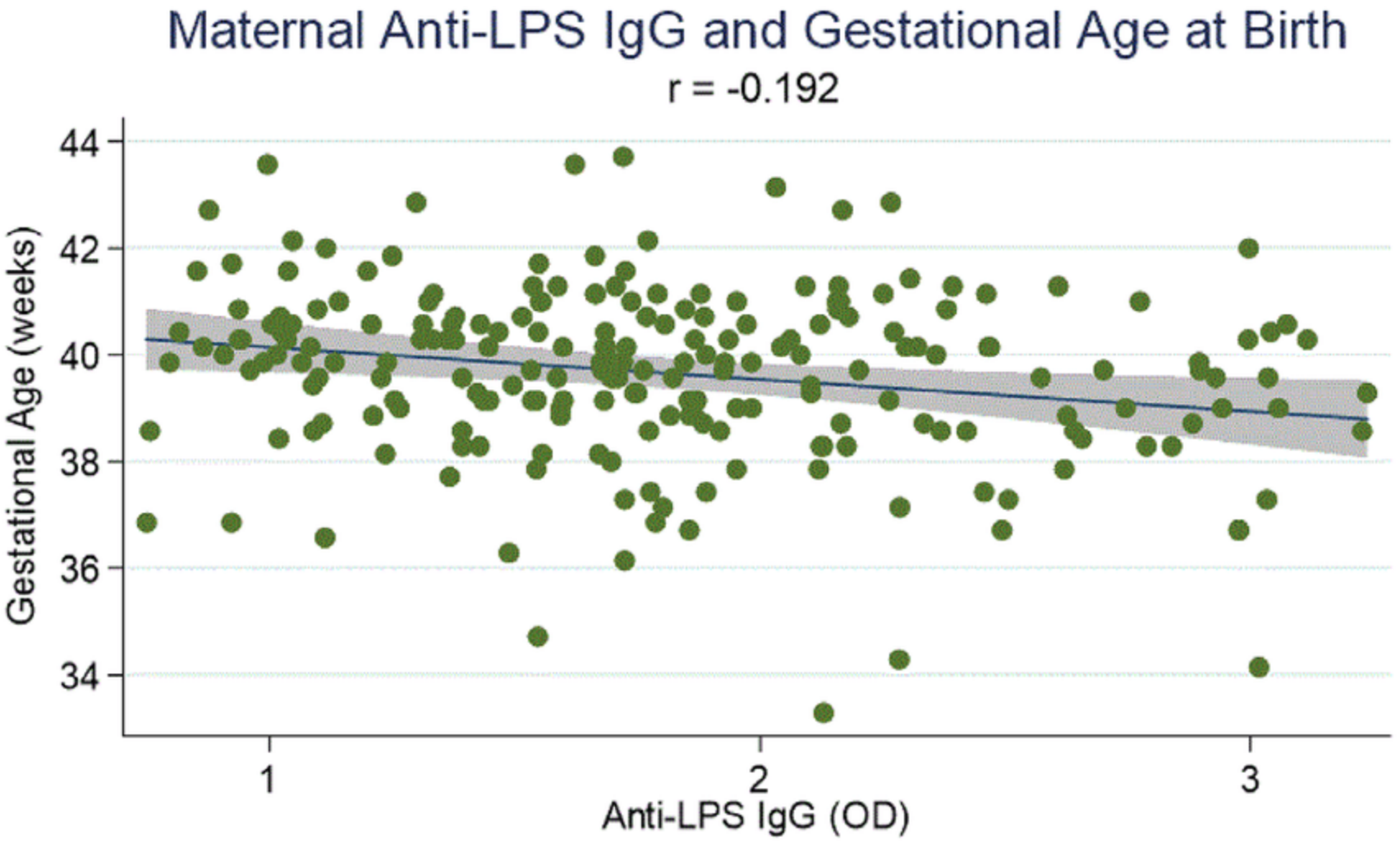
**TABLE 3**

Biomarkers of maternal EED as predictors of infant gestational age (weeks), length (centimeters), and LAZ at birth ($n = 220$) in unadjusted and adjusted linear regression models[1]

| | Gestational age, wk | | Length, cm | | LAZ | |
|---|---|---|---|---|---|---|
| | Unadjusted | Adjusted | Unadjusted | Adjusted | Unadjusted | Adjusted |
| L:M | 0.04 (−0.22, 0.30) $P = 0.761$ | 0.02 (−0.24, 0.29) $P = 0.858$ | 0.04 (−0.21, 0.30) $P = 0.746$ | 0.01 (−0.22, 0.24) $P = 0.901$ | 0.03 (−0.11, 0.16) $P = 0.712$ | 0.01 (−0.11, 0.13) $P = 0.842$ |
| %LE | 0.02 (−0.25, 0.29) $P = 0.897$ | 0.006 (−0.27, 0.28) $P = 0.968$ | −0.03 (−0.29, 0.24) $P = 0.850$ | −0.03 (−0.27, 0.20) $P = 0.776$ | −0.008 (−0.15, 0.13) $P = 0.915$ | −0.009 (−0.13, 0.11) $P = 0.881$ |
| Anti-flagellin IgA | −0.26 (−0.96, 0.44) $P = 0.463$ | −0.37 (−1.10, 0.36) $P = 0.322$ | 0.11 (−0.57, 0.79) $P = 0.743$ | −0.15 (−0.79, 0.49) $P = 0.643$ | 0.05 (−0.31, 0.41) $P = 0.785$ | −0.11 (−0.44, 0.23) $P = 0.533$ |
| Anti-LPS IgA | −0.24 (−1.06, 0.58) $P = 0.566$ | −0.25 (−1.10, 0.60) $P = 0.564$ | −0.36 (−1.15, 0.43) $P = 0.372$ | −0.48 (−1.22, 0.25) $P = 0.195$ | −0.21 (−0.63, 0.21) $P = 0.323$ | −0.28 (−0.67, 0.10) $P = 0.152$ |
| Anti-flagellin IgG | −0.79 (−1.66, 0.08) $P = 0.075$ | −0.89 (−1.77, −0.01) $P = 0.047$* | −0.68 (−1.52, 0.16) $P = 0.110$ | −0.80 (−1.55, −0.05) $P = 0.036$* | −0.38 (−0.83, 0.06) $P = 0.089$ | −0.44 (−0.83, −0.05) $P = 0.029$* |
| Anti-LPS IgG | −0.98 (−1.82, −0.15) $P = 0.021$* | −1.01 (−1.87, −0.17) $P = 0.019$* | −0.50 (−1.32, 0.32) $P = 0.234$ | −0.79 (−1.54, −0.04) $P = 0.039$* | −0.29 (−0.72, 0.15) $P = 0.197$ | −0.40 (−0.79, −0.01) $P = 0.043$* |

[1] Values are $\beta$-coefficients (95% CIs) and $P$ values; all EED biomarkers were natural log transformed before analysis. Adjusted model controls for maternal age, height, diastolic blood pressure, years of education, first pregnancy (yes/no), *$P < 0.05$. Household Food Insecurity Access Scale score, safe water (yes/no), and infant birth weight. EED; environmental enteric dysfunction; LAZ, length-for-age $z$ score; L:M, lactulose:mannitol; %LE, percentage lactulose excretion.

In adjusted linear regression models, higher ln concentrations of anti-flagellin IgG and anti-LPS IgG were significantly associated with shorter length of infant gestational age at birth, lower length at birth, and lower LAZ at birth


Maternal Anti-LPS IgG and Gestational Age at Birth
r = -0.192
Gestational Age (weeks)
44
42
40
38
36
34
1
2
3
Anti-LPS IgG (OD)

# Next steps

- UBCS maternal samples from pregnancy analyzed for anti-flagellin and anti-LPS

- What is the relationship between maternal EED biomarkers (anti-flagellin and anti-LPS Igs) and infant birth outcomes, growth (6 months), and EED status (6 months)?

# Questions?