EFFECT OF EARLY-LIFE UNDERNUTRITION ON THE GUT MICROBIOTA

Geoffrey A. Preidis, M.D., Ph.D.
Assistant Professor of Pediatrics
Section of Gastroenterology, Hepatology, and Nutrition
Department of Pediatrics
Baylor College of Medicine
Texas Children’s Hospital
THE #1 HEALTH PROBLEM PLAGUING CHILDREN TODAY

- Globally, undernutrition contributes to **3.1 million child deaths per year**.
- 96 million children (14%) are underweight and 159 million (24%) are stunted.
A “VICIOUS CYCLE” OF GI PATHOPHYSIOLOGIES

- Enteric dysfunction, malabsorption → increased fermentation substrates
- Low-protein diet, hypoalbuminemia → ascites
- Liver function anomalies → steatosis, hepatomegaly, ascites → decreased bile acid synthesis, impaired weight gain → coagulopathy
- Gastrointestinal dysmotility → luminal stasis, fecal impaction, poor appetite
- Infection or microbial “dysbiosis” → inflammation, gas, bloating
- “Thrifty phenotype” → increased risk of obesity and metabolic diseases
CASE PRESENTATION

Weight (kg)

[Graph showing weight gain over years]

Texas Children's Pediatrics
READY-TO-USE THERAPEUTIC FOOD (RUTF)

- Nut paste, sugar, vegetable oil, milk powder, vitamins & minerals
- Can be expensive, often must be imported
- Long-term outcomes with respect to child growth have yielded mixed results in meta-analyses


http://www.thp.org/plumpynut-a-cure-for-malnutrition
CASE PRESENTATION
THE VICIOUS CYCLE OF REPEATED INFECTIONS AND EARLY UNDERNUTRITION

OBJECTIVES

1. Recognize the distinct patterns of gut bacterial community configurations in undernourished children

2. List dietary, environmental, and host factors that shape the gut microbiome of undernutrition

3. Evaluate the clinical evidence supporting the use of microbiome-targeting therapies to enhance growth
Gastric/duodenal bacterial overgrowth was subsequently reported in undernourished children from:

- **Guatemala**
  Dammin GJ. *Bull World Health Organ* 1964;31:29-32.

- **Aboriginal Australia**

- **Indonesia**

- **Brazil**

- **The Gambia**
SEVERELY UNDERNOURISHED (BUT OTHERWISE HEALTHY) CHILDREN HAVE ABNORMAL INTESTINAL HISTOLOGY

Dammin GJ. *Bull World Health Organ* 1964;31:29-32.
STOOL FROM UNDERNOURISHED VS HEALTHY CHILDREN HAS DECREASED MICROBIOTA RICHNESS

ANOREXIA NERVOSA PATIENTS ALSO HAVE “DYSBIOSIS” WITH DECREASED DIVERSITY

Proteobacteria, including Enterobacteriaceae, are overrepresented in anorexia vs healthy controls

BETA DIVERSITY IN UNDERNOURISHED VS HEALTHY CHILDREN

• Increased abundance of pathogenic genera within the phylum Proteobacteria, including Enterobacter, Escherichia, Klebsiella, and Shigella, even in the absence of diarrhea.

- Decreased abundance of genera with potentially beneficial microbes, including Roseburia, Faecalibacterium, Butyrivibrio, Lactobacillus, and Bifidobacterium.

GUT MICROBIOTA MATURITY IS IMPAIRED IN CHILD UNDERNUTRITION

KEY POINT 1

Objective 1: Recognize the distinct patterns of gut bacterial community configurations in undernourished children

- Children who are undernourished from a variety of causes have gut microbial community alterations ("dysbiosis"), characterized by:
  - Decreased richness (number of unique taxa)
  - Increased abundance of pathogens & potential pathogens
  - Decreased abundance of potentially beneficial microbes
  - Delayed microbiome maturation
OBJECTIVES

1. Recognize the distinct patterns of gut bacterial community configurations in undernourished children

2. List dietary, environmental, and host factors that shape the gut microbiome of undernutrition

3. Evaluate the clinical evidence supporting the use of microbiome-targeting therapies to enhance growth
Factors that may cause intestinal microbial dysbiosis in undernourished children:

- Maternal & perinatal factors
  - Dietary monotony, limited variation of nutrient-poor foods
  - Decreased production of ileal antimicrobial peptides
  - Enteropathogens (toxins, nutrient competition, mucus layer changes, altered motility)
  - Inflammation (antimicrobial peptides, disrupted O₂ gradient, reactive O₂ & N₂ species)
  - Impaired innate & acquired immunity exacerbating infection intensity & damage
  - Altered bile acid biosynthesis

Microbial “dysbiosis”

- Decreased richness & commensal: pathogen ratio, Delayed maturation

Mechanisms by which intestinal microbial dysbiosis may impair weight gain:

- Toxins & other virulence factors
  - Enhanced pathogen colonization & invasion
  - Promotion of inflammation & enteric dysfunction
  - Less efficient energy harvest from non-digestible dietary components
  - Impaired de novo micronutrient biosynthesis
  - Differential metabolism of primary & conjugated bile acids
  - Altered transit time, nutrient malabsorption, decreased appetite, barrier disruption
  - Changes in systemic metabolites, hormones, and somatotropic axis

Impaired weight gain

Persistent microbial dysbiosis

Undernutrition

Stunting, decreased fitness & earning potential

Impaired vaccine responses

Increased risk of obesity & co-morbidities

Altered brain metabolites, cognitive impairment

INFLAMMATION (A FEATURE OF ENVIRONMENTAL ENTEROPATHY) ALTERS THE GUT MICROBIOME

1. By triggering an immune response in which antimicrobial peptides released into the lumen innately defend against pathogens, but also target subsets of commensals

   Sanchez de Medina.. Martinez-Augustin. *Inflamm Bowel Dis* 2014;20:2394-404

2. By disrupting the tightly regulated oxic-microoxic-anoxic zones in the lumen, influencing bacterial growth and transcriptional programs


3. By generating reactive oxygen and nitrogen species, which shape microbial populations by facilitating respiration among certain bacteria

Compared to healthy Italian children, stool from healthy children in Burkina Faso was enriched with microbes (e.g., *Prevotella*, *Xylanibacter*) that harbor enzymes for metabolizing non-digestible dietary cellulose and xylans, key components of the Burkina Faso diet.

Fig. 1. Life in a rural village of Burkina Faso. (A) Village of Boulpon. (B) Traditional Mossi dwelling. (C) Map of Burkina Faso (modified from the United States CIA’s World Factbook, 34). (D) Millet and sorghum (basic components of Mossi diet) grain and flour in typical bowls. (E) Millet and sorghum is ground into flour on a grinding stone to produce a thick porridge called Tô.

Compared to formula-fed infants, the gut microbiota of breastfed infants is less diverse, consistent with enrichment of genes required for the degradation of human milk oligosaccharides (HMOs) from breast milk.
DIET INFLUENCES THE INFANT MICROBIOME

- Not all breast milk is equal
- Malawian mothers with severely stunted vs healthy infants produced decreased quantities of human milk oligosaccharides (HMOs)
- How might HMOs affect growth?

DIET INFLUENCES THE INFANT MICROBIOME

Germ-free mice

Prototypic Malawian Diet + Sialylated Milk Oligosaccharides

No growth effects

DIET-INDUCED GUT MICROBIAL “DYSBIOSIS” CAN CONFER SUSCEPTIBILITY TO INFECTION

**UNDERNOURISHED MOUSE PUPS HAVE DECREASED FECAL MICROBIAL DIVERSITY, WITH INCREASED ABUNDANCE OF MUCOLYTIC BACTERIA**

In humans, the relative abundance of *Akkermansia muciniphila* is inversely proportional to body mass index (BMI).


THIS OVERABUNDANCE OF MUCOLYTIC MICROBES IS ASSOCIATED WITH INCREASED HOST MUCIN GENE EXPRESSION

**Muc2**

Control Pups  Malnourished Pups

*Conventional*

Control Pups  Malnourished Pups

*Germ Free*

*p < 0.05*
THREE MOUSE MODELS OF EARLY-LIFE UNDERNUTRITION

• Timed Separation (TmSep)
  • TmSep: 12 hours/day away from mothers
  • Controls: litters of normal pups

• Regional Basic Diet (RBD)
  • RBD: Mothers fed 5% fat, 7% protein, 88% carb
  • Controls: Mothers fed isocaloric diet

• RBD Young Adults
  • RBD Pups weaned to RBD chow
  • Controls: Control Pups weaned to isocaloric Control Diet
Slow transit in undernutrition is linked to bacterial overgrowth, abdominal distention, constipation, and blunted appetite.

GI transit time influences microbiome composition and function.

UNDERNUTRITION SLOWS GASTRIC AND SMALL BOWEL TRANSIT IN MULTIPLE MOUSE MODELS

Body weight, g

Control  RBD  Control  RBD

Body weight, g

Control  RBD  TmSep

GE = gastric emptying
MGC = mean geometric center of bolused dye

Transit time in undernourished young adult males was minimally affected.
Decreased bile acids in undernutrition are linked to dietary fat malabsorption, fat-soluble vitamin deficiencies, bacterial overgrowth, and poor weight gain during refeeding.

Intestinal bile acids help regulate gut microbial populations.

UNDERNOURISHED MICE (AND CHILDREN) EXHIBIT MACROVESICULAR STEATOSIS

Control

Oil red O stain, flash-frozen livers, x160

RBD Young Adults
UNDERNOURISHED MICE HAVE NO PEROXISOMES
Altered Intestinal Microbiota?
Factors that may cause intestinal microbial dysbiosis in undernourished children

Maternal & perinatal factors
- Dietary monotony, limited variation of nutrient-poor foods
- ↓ ratio of dietary:endogenous glycans available for metabolism
- Decreased production of ileal antimicrobial peptides
- Enteropathogens (toxins, nutrient competition, mucus layer changes, altered motility)
- Inflammation (antimicrobial peptides, disrupted $O_2$ gradient, reactive $O_2$ & $N_2$ species)
- Impaired innate & acquired immunity exacerbating infection intensity & damage
- Altered bile acid biosynthesis

Microbial “dysbiosis”
- Decreased richness & commensal: pathogen ratio, Delayed maturation

Mechanisms by which intestinal microbial dysbiosis may impair weight gain
- Toxins & other virulence factors
- Enhanced pathogen colonization & invasion
- Promotion of inflammation & enteric dysfunction
- Less efficient energy harvest from non-digestible dietary components
- Impaired de novo micronutrient biosynthesis
- Differential metabolism of primary & conjugated bile acids
- Altered transit time, nutrient malabsorption, decreased appetite, barrier disruption
- Changes in systemic metabolites, hormones, and somatotropic axis

Impaired weight gain

Persistent microbial dysbiosis

Undernutrition

Stunting, decreased fitness & earning potential

Impaired vaccine responses

Increased risk of obesity & co-morbidities

Altered brain metabolites, cognitive impairment
GERM-FREE MICE TOLERATE EARLY-LIFE UNDERNUTRITION BETTER THAN CONVENTIONAL MICE WITH INTESTINAL BACTERIA

Δ Weight From P5 to P10 (g)

Control  TmSep

Conventional

n = 10-18
FECAL MICROBES FROM UNDERNOURISHED CHILDREN CAN CAUSE UNDERNUTRITION IN GNOTOBIOTIC MICE (UNDER THE RIGHT CONDITIONS)

Diet is key!

GUT BACTERIA (EVEN NON-PATHOGENS) CAN CAUSE INFLAMMATION AND GROWTH IMPAIRMENT

Consumption of a low-protein, low-fat diet, in combination with iterative exposure to 6 non-pathogenic gut microbes, produces inflammation and weight loss without overt diarrhea.

Factors that may cause intestinal microbial dysbiosis in undernourished children

Maternal & perinatal factors
- Dietary monotony, limited variation of nutrient-poor foods
- ↓ ratio of dietary:endogenous glycans available for metabolism
- Decreased production of ileal antimicrobial peptides
- Enteropathogens (toxins, nutrient competition, mucus layer changes, altered motility)
- Inflammation (antimicrobial peptides, disrupted \( \text{O}_2 \) gradient, reactive \( \text{O}_2 \) & \( \text{N}_2 \) species)
- Impaired innate & acquired immunity exacerbating infection intensity & damage
- Altered bile acid biosynthesis

Undernutrition

Impaired weight gain

Persistent microbial dysbiosis

Microbial “dysbiosis”

Decreased richness & commensal: pathogen ratio, Delayed maturation

Mechanisms by which intestinal microbial dysbiosis may impair weight gain

Toxins & other virulence factors
- Enhanced pathogen colonization & invasion
- Promotion of inflammation & enteric dysfunction
- Less efficient energy harvest from non-digestible dietary components
- Impaired de novo micronutrient biosynthesis
- Differential metabolism of primary & conjugated bile acids
- Altered transit time, nutrient malabsorption, decreased appetite, barrier disruption
- Changes in systemic metabolites, hormones, and somatotropic axis

Stunting, decreased fitness & earning potential
- Impaired vaccine responses
- Increased risk of obesity & co-morbidities
- Altered brain metabolites, cognitive impairment

Objective 2: List dietary, environmental, and host factors that shape the gut microbiome of undernutrition

• “Dysbiosis” of undernutrition can be shaped by many factors, including:
  • Prenatal/perinatal factors, carbohydrate composition of breast milk or the post-wean diet, inflammation, presence of pathogens, host intestinal mucus profile...

• Mechanisms by which “dysbiosis” can impair weight gain are less clear, but might include:
  • Bacterial toxins, subclinical inflammation, decreased efficiency of energy harvest from diet, impaired micronutrient biosynthesis, altered gastrointestinal motility, bile acid pool changes...
OBJECTIVES

1. Recognize the distinct patterns of gut bacterial community configurations in undernourished children

2. List dietary, environmental, and host factors that shape the gut microbiome of undernutrition

3. Evaluate the clinical evidence supporting the use of microbiome-targeting therapies to enhance growth
A PROMISING TRIAL OF ANTIBIOTICS FOR CHILDREN WITH SEVERE ACUTE UNDERNUTRITION

• 2,767 Malawian children prescribed RUTF as outpatient treatment for severe acute undernutrition

• Children randomly assigned to twice-daily placebo vs amoxicillin (80-90 mg/kg/day) or cefdinir (14 mg/kg/day) for 7 days

• **Placebo** increased the relative risk of **treatment failure**:
  • RR 1.32 [1.04 – 1.68] vs amoxicillin
  • RR 1.64 [1.27 – 2.11] vs cefdinir

• **Placebo** increased the relative risk of **mortality**:
  • RR 1.55 [1.07 – 2.24] vs amoxicillin
  • RR 1.80 [1.22 – 2.64] vs cefdinir

TWO OTHER LARGE TRIALS FAILED TO SHOW BENEFIT OF ANTIBIOTICS FOR UNDERNOURISHED CHILDREN

• 2,412 children in Niger with severe acute undernutrition randomized to twice-daily placebo vs amoxicillin (80 mg/kg/day) x7 days
  • No effect on nutritional recovery over 8 week follow-up

• 1,778 children in Kenya who had recovered from severe acute undernutrition randomized to daily placebo vs co-trimoxazole (120 or 240 mg/day) x6 months
  • No effect on mortality over 12 month follow-up

THE PRONUT STUDY: TESTING A PREBIOTIC + PROBIOTIC IN CHILD UNDERNUTRITION

- Randomized, placebo controlled trial enrolled 795 Malawian children hospitalized for nutritional rehabilitation
- Children randomized to RUTF + placebo vs RUTF + Synbiotic 2000 Forte
  - 4 probiotics: *Pediococcus pentosaceus, Leuconostoc mesenteroides, Lactobacillus paracasei, Lactobacillus plantarum*
  - 4 prebiotics: oat bran, inulin, pectin, resistant starch
- Median 33 days of treatment

THE PRONUT STUDY: TESTING A PREBIOTIC + PROBIOTIC IN CHILD UNDERNUTRITION

• Result: No significant effect on nutritional cure or on any other nutritional outcome

• Reasons for this negative result?

Synbiotic 2000 Forte

• 4 probiotics: *Pediococcus pentosaceus*, *Leuconostoc mesenteroides*, *Lactobacillus paracasei*, *Lactobacillus plantarum*

• 4 prebiotics: oat bran, inulin, pectin, resistant starch
SUMMARY OF LARGE RANDOMIZED, PLACEBO-CONTROLLED CLINICAL TRIALS TO DATE

Table 1. Randomized controlled trials that evaluate microbiome-targeting therapies to improve nutritional status in undernourished children.

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Setting</th>
<th>Number of Study Participants</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synbiotic 2000 Forte</td>
<td>Malawi</td>
<td>795</td>
<td>No significant effect on nutritional cure</td>
<td>104</td>
</tr>
<tr>
<td>Amoxicillin or cefdinir</td>
<td>Malawi</td>
<td>2767</td>
<td>Placebo increased risk of treatment failure (RR 1.32 [1.04–1.68] vs amoxicillin; RR 1.64 [1.27–2.11] vs cefdinir) and mortality (RR 1.55 [1.07–2.24] vs amoxicillin; RR 1.80 [1.22–2.64] vs cefdinir)</td>
<td>105</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>Niger</td>
<td>2412</td>
<td>No significant effect on nutritional recovery</td>
<td>106</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>Kenya</td>
<td>1778</td>
<td>No significant effect on mortality</td>
<td>107</td>
</tr>
</tbody>
</table>

Note. RR, relative risk followed by 95% confidence interval in brackets.

- None of these landmark studies assessed how treatment vs placebo affected the gut microbiome
- Would a beneficial effect of a broad-spectrum antibiotic be worth the risks?
KEY POINT 3

Objective 3: Evaluate the clinical evidence supporting the use of microbiome-targeting therapies for undernutrition

- Although there is currently not enough clinical evidence to recommend microbiome-targeting therapies for undernourished children, promising preclinical models suggest that individualized therapies might one day allow clinicians to improve a child’s growth trajectory
WHAT LIES AHEAD?

Development of low-cost biomarkers to:
A) Identify children who would benefit from microbiome-targeting therapies
B) Select the specific agents needed to address an individual’s functional imbalances

Graph showing weight gain over years with a child undernourished with gut microbial "dysbiosis" and possible restoration of a healthy microbiota.
ACKNOWLEDGEMENTS

Funding

- Young Investigator Grant for Probiotics Research, Global Probiotics Council
- Early Career Award, Thrasher Research Fund
- Pediatric GI Training Grant, NIH/NIDDK T32DK007664 (PI: Shulman)
- NASPGHAN Foundation / Nestlé Nutrition Research Young Investigator Development Award
- Pilot/Feasibility Award, Texas Medical Center Digestive Disease Center (PHS grant P30DK56338)
- Functional and Mechanistic Award, Alkek Center for Metagenomics and Microbiome Research
- Research Grant, American Neurogastroenterology and Motility Society
- AGA-Rome Foundation Functional GI & Motility Disorders Pilot Research Award
- Chao Physician-Scientist Award
- NIH/NIDDK K08DK113114

Baylor College of Medicine
Pediatric GI, Hepatology & Nutrition Research Laboratories

- Tripti Halder
- Sanjiv Harpavat
- Swapna Krishnamoorthy
- Subapradha Narayanan
- Benjamin Shneider
- Krishnakant Soni
- Sundararajah Thevananther
- M. Elizabeth Tessier
- Jennifer Yeh

Moore Laboratory

- Sungwoo Choi
- Kangho Kim
- David Moore

Robert Britton
- Rui Chen
- Cristian Coarfa
- Margaret Conner
- Sridevi Devaraj
- Milton Finegold
- Robert Shulman
- Jennifer Spinler
- Lanlan Shen
- Arun Sreekumar
- James Versalovic
- Lisa White

Moore Laboratory

- Sungwoo Choi
- Kangho Kim
- David Moore

Robert Britton
- Rui Chen
- Cristian Coarfa
- Margaret Conner
- Sridevi Devaraj
- Milton Finegold
- Robert Shulman
- Jennifer Spinler
- Lanlan Shen
- Arun Sreekumar
- James Versalovic
- Lisa White