EFFECT OF EARLY-LIFE UNDERNUTRITION ON THE GUT MICROBIOTA

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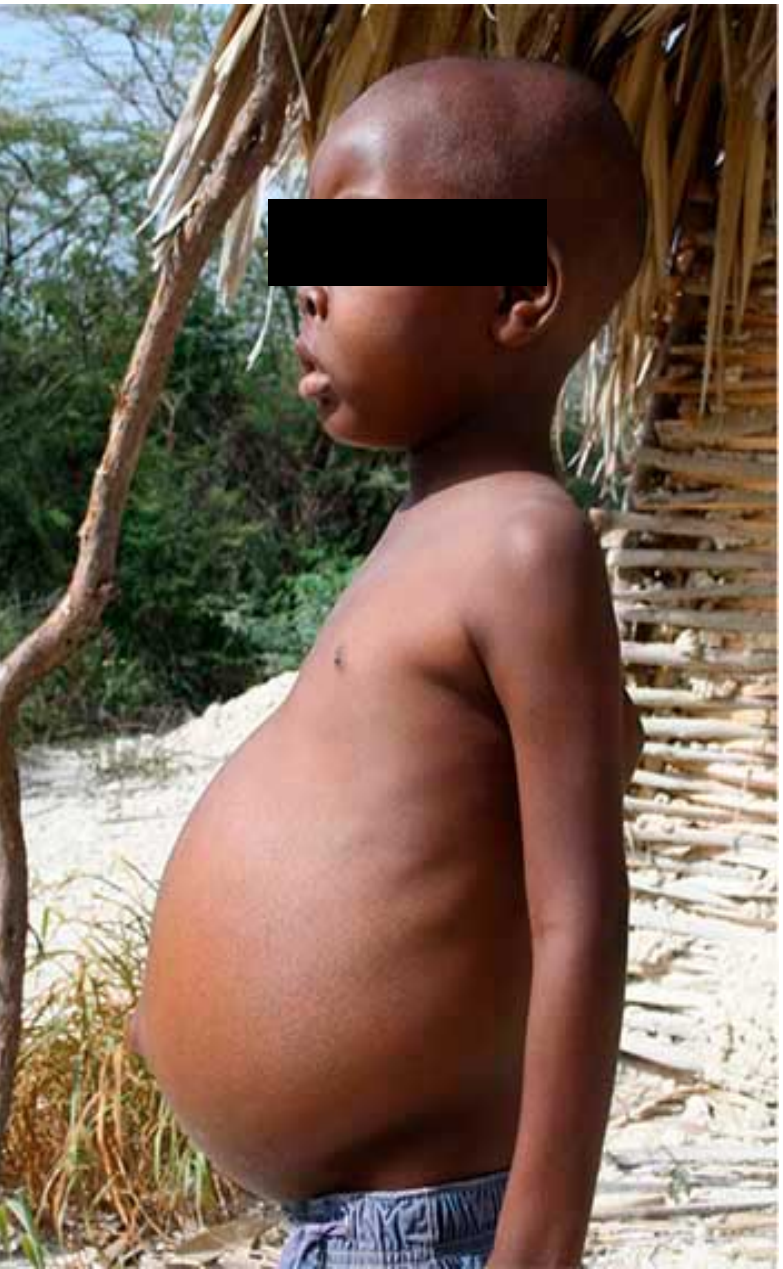
THE #1 HEALTH PROBLEM PLAGUING CHILDREN TODAY

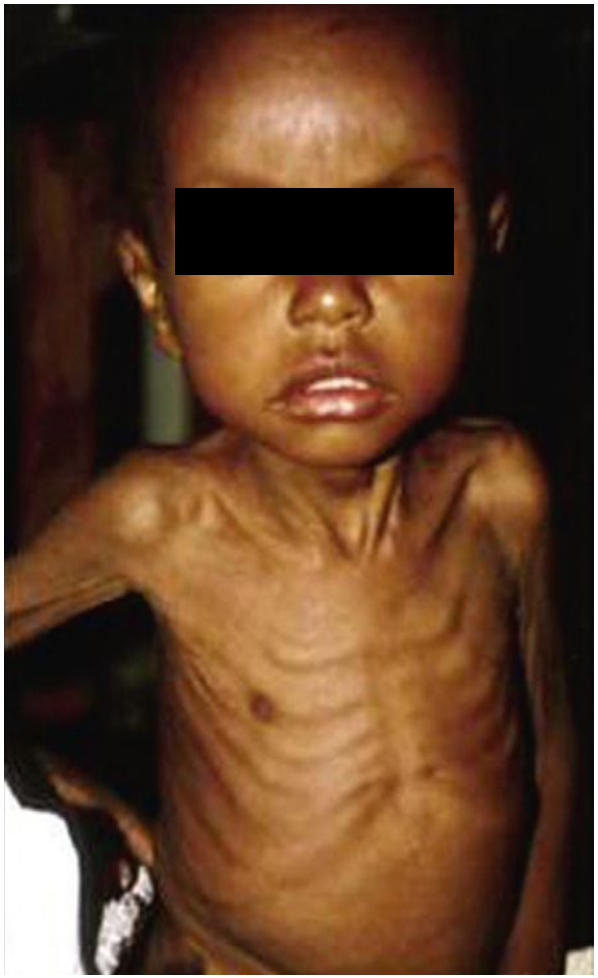
- Globally, undernutrition contributes to **3.1 million child deaths per year**.
- 96 million children (14%) are underweight and 159 million (24%) are stunted.

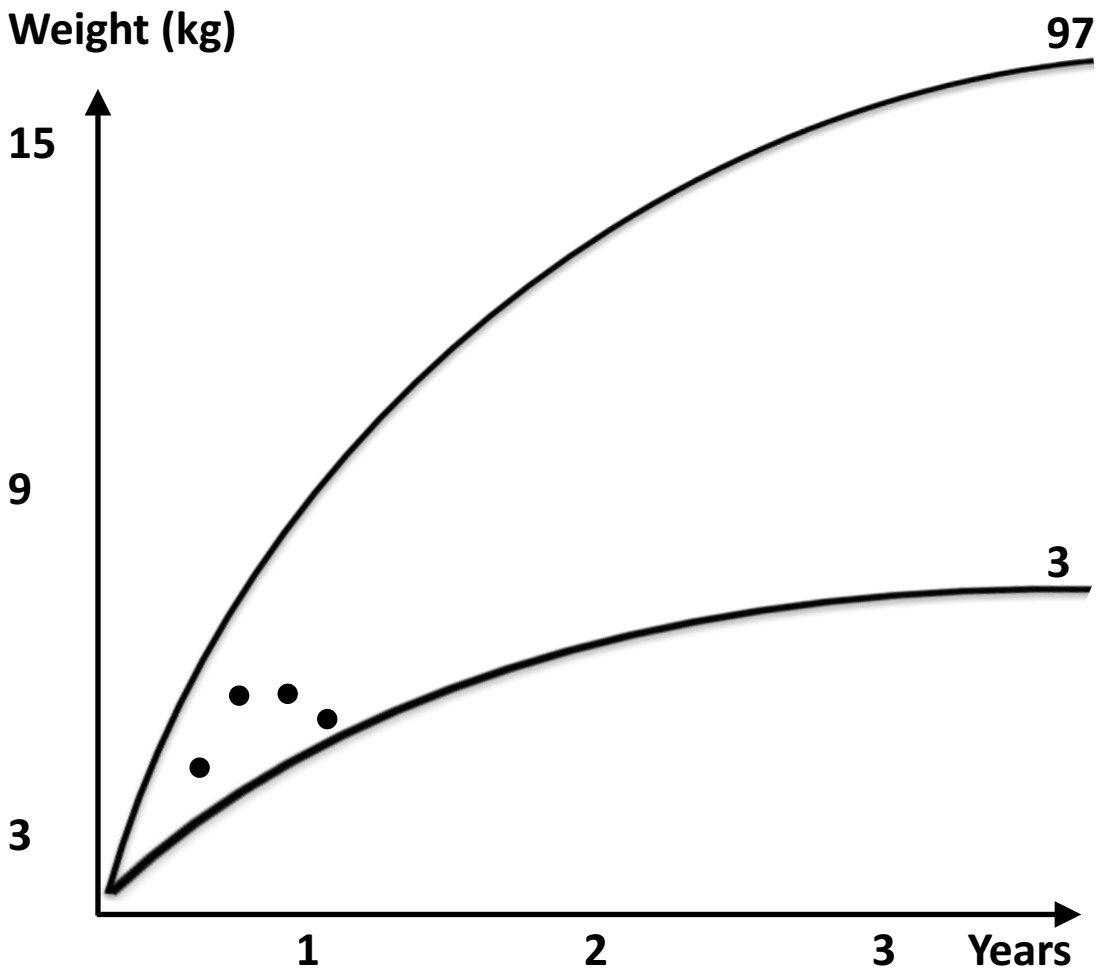
A "VICIOUS CYCLE" OF GI PATHOPHYSIOLOGIES



- 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





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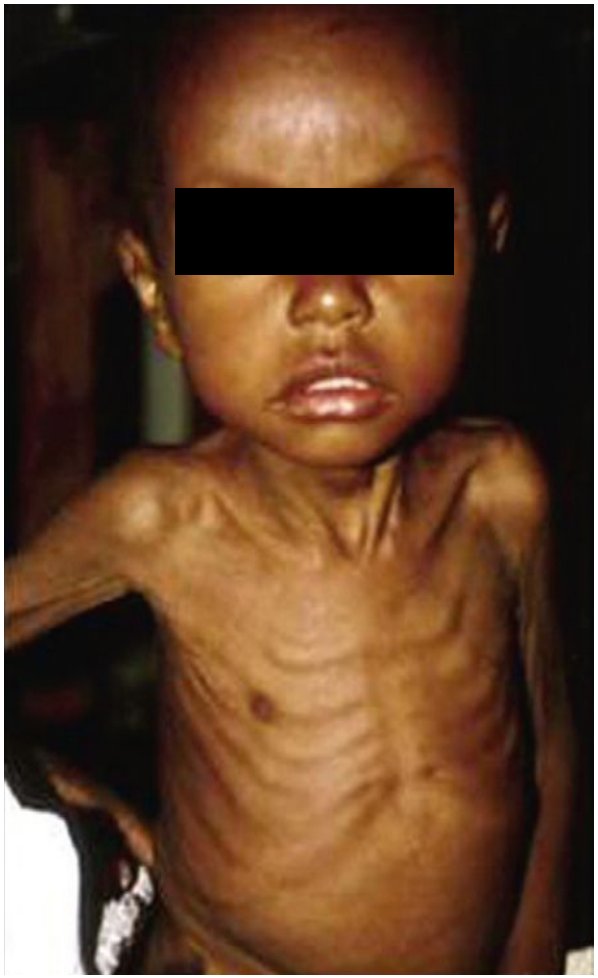
READY-TO-USE THERAPEUTIC FOOD (RUTF)

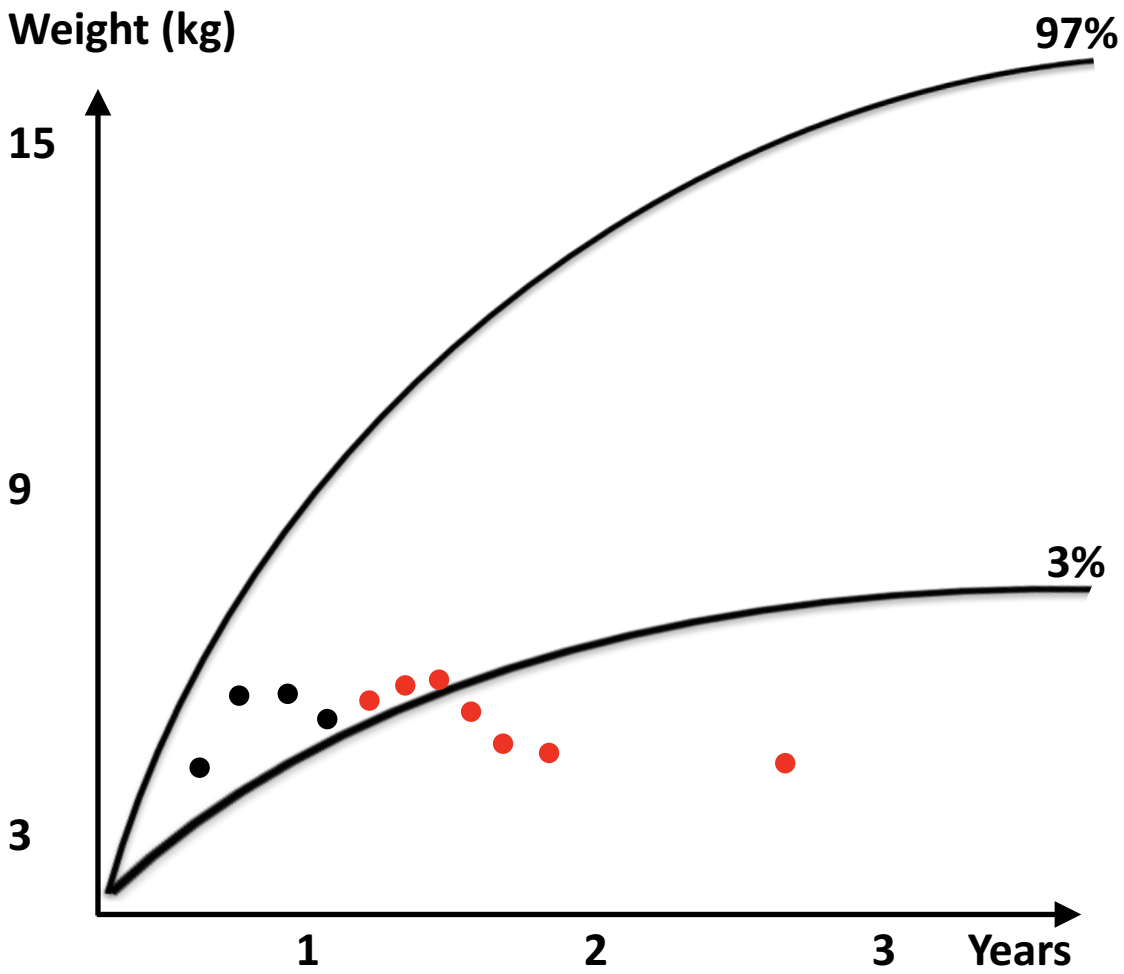
- Nut paste, sugar, vegetable oil, milk powder, vitamins & minerals
- Can be expensive, often must be imported
- Long-term outcomes with respect to child growth have yielded mixed results in meta-analyses

http://www.thp.org/plumpynut-a-cure-for-malnutrition

Ashworth. *Food Nutr Bull* 2006;27:S24-48.
Lenters.. Bhutta. *BMC Public Health* 2013;13:1-15.
Schoones.. Volmink. *Cochrane Database Syst Rev* 2013;6:1-90.

CASE PRESENTATION





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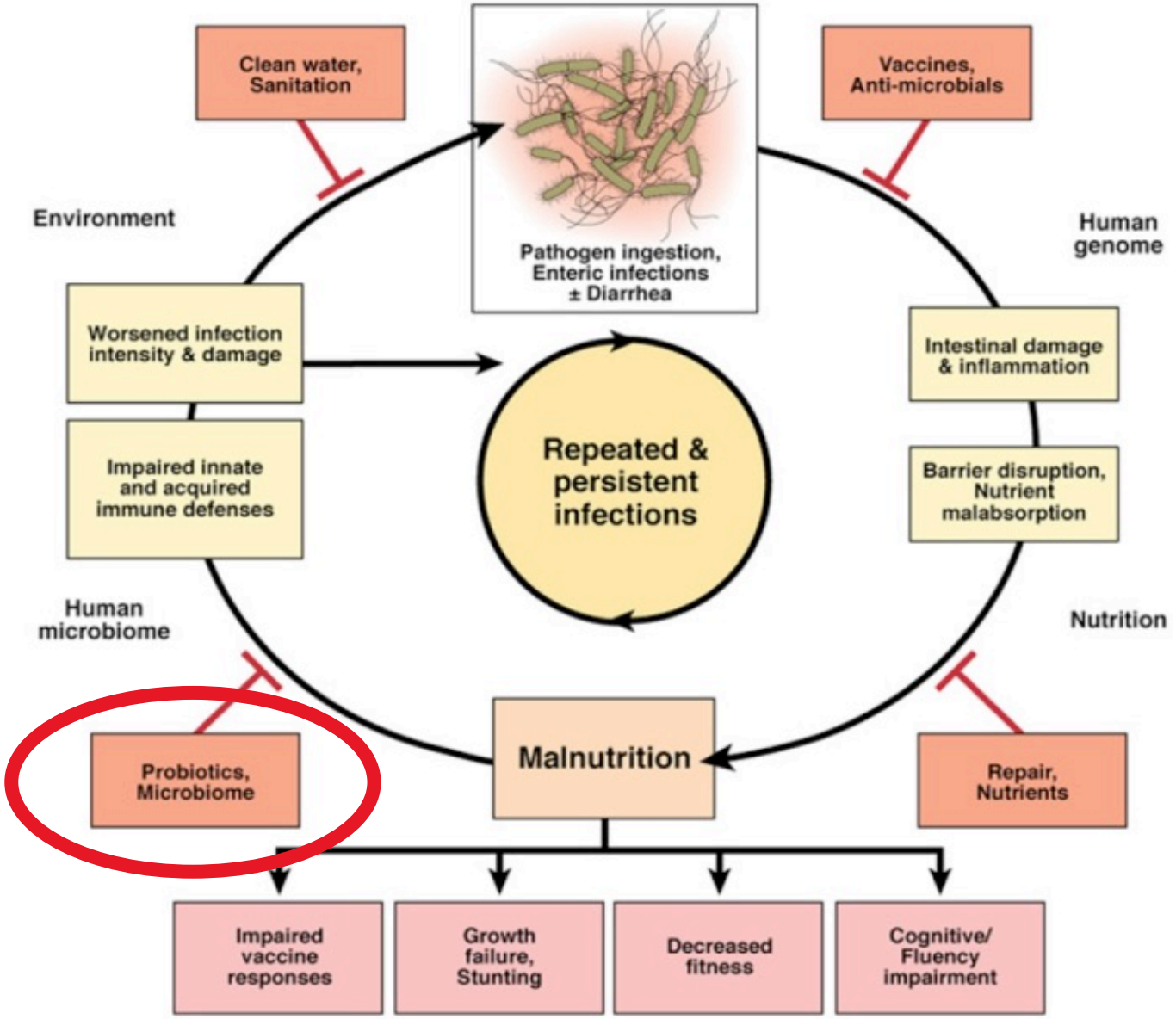
THE VICIOUS CYCLE OF REPEATED INFECTIONS AND EARLY UNDERNUTRITION



Preidis.. Versalovic. *Gastroenterol* 2011;140:8-14.

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

CHANGES IN INTESTINAL BACTERIAL FLORA AND ROLE OF INFECTION IN KWASHIORKOR

P. M. SMYTHE
M.B. Cantab., M.R.C.P.

DEPARTMENT OF CHILD HEALTH, UNIVERSITY OF CAPE TOWN AND GROOTE SCHUUR HOSPITAL

Gastric/duodenal bacterial overgrowth was subsequently reported in undernourished children from:

- **Guatemala**
  Dammin GJ. *Bull World Health Organ* 1964;31:29-32.
  Mata.. Viteri. *Am J Clin Nutr* 1972;25:118-26.
- **Aboriginal Australia**
  Gracey & Stone. *Aust N Z J Med* 1972;2:215-9.
- **Indonesia**
  Gracey & Stone. *Am J Clin Nutr* 1973;26:1170-4.
- **Brazil**
  Maffei & Nobrega. *Gut* 1975;16:719-26.
- **The Gambia**
  Heyworth & Brown. *Arch Dis Child* 1975;50:27-33.

BACTERIAL GROWTH FROM GASTRIC JUICE, AND RECTAL SWAB, AND EFFECT OF THREE DAYS' ANTIBIOTIC TREATMENT ON THE INTESTINAL FLORA, IN 20 PATIENTS WITH KWASHIORKOR

Organisms	Gastric juice	Rectal swab	Both gastric juice and rectal swab	Rectal swab after 3 days' antibiotics
Staph. aureus	10	8	6	0
Coliforms	11	17	11	5
Salmonella	1	3	1	1
Shigella flexneri	0	2	0	0
Paracolon	2	5	1	4
Enterococci	0	4	0	7
Proteus morgani	0	14	0	4
Ps. aeruginosa	0	0	0	6
Extent of bacterial growth:				
None	3	0	..	5
Scanty	10	0	..	12
Moderate	6	0	..	3
Heavy	1	20	..	0

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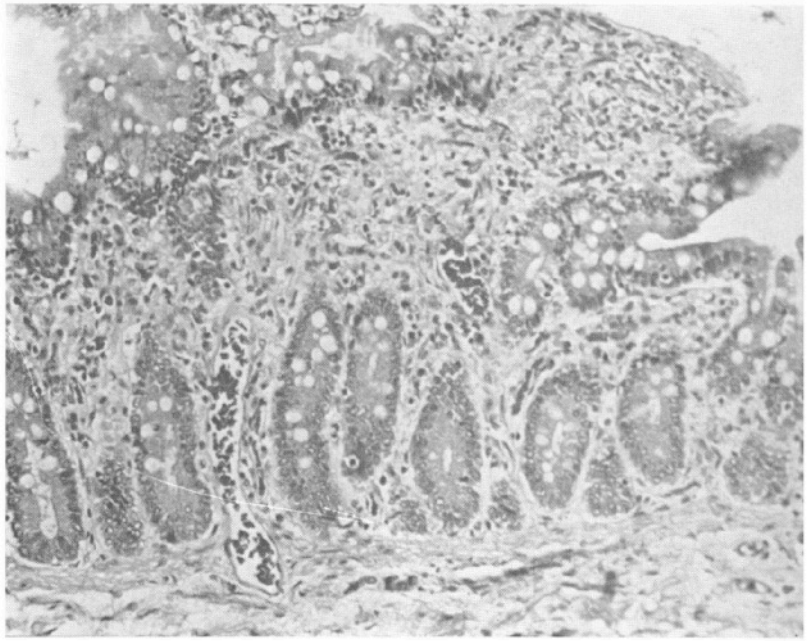
Smythe PM. *Lancet* 1958;2:724-7.

SEVERELY UNDERNOURISHED (BUT OTHERWISE HEALTHY) CHILDREN HAVE ABNORMAL INTESTINAL HISTOLOGY



1. Section of jejunum obtained from Guatemalan child with malnutrition but no diarrhoea. The structure of the villi is distorted and there is a reduced ratio of villus height to crypt depth. Note the sparse cellularity of the lamina propria and the complement of goblet cells.

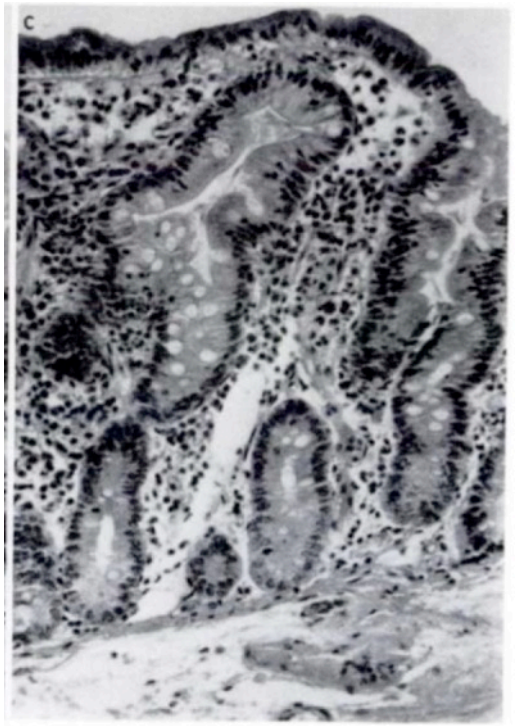


FIG. 2. Progressive fusion of the tips of two villi

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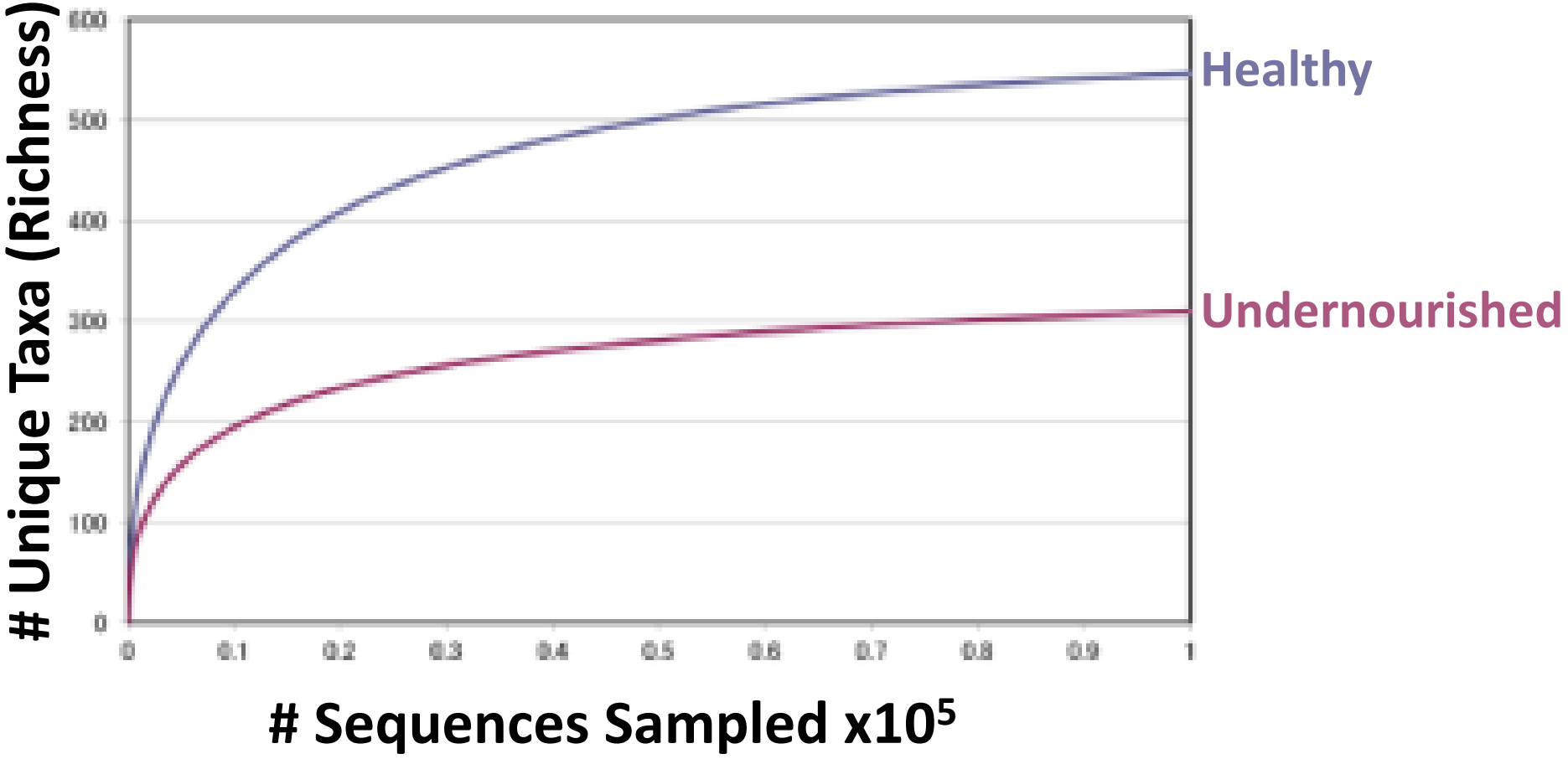
Dammin GJ. *Bull World Health Organ* 1964;31:29-32.
Schneider & Viteri. *Am J Clin Nutr* 1972;25:1092-102.

STOOL FROM UNDERNOURISHED VS HEALTHY CHILDREN HAS DECREASED MICROBIOTA RICHNESS



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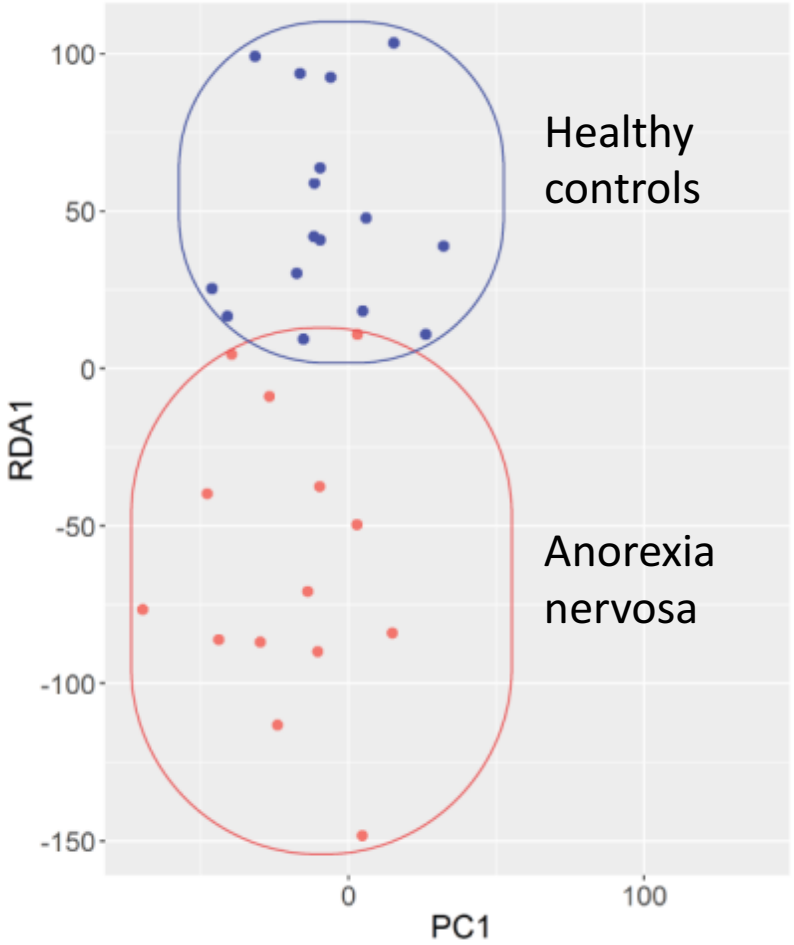
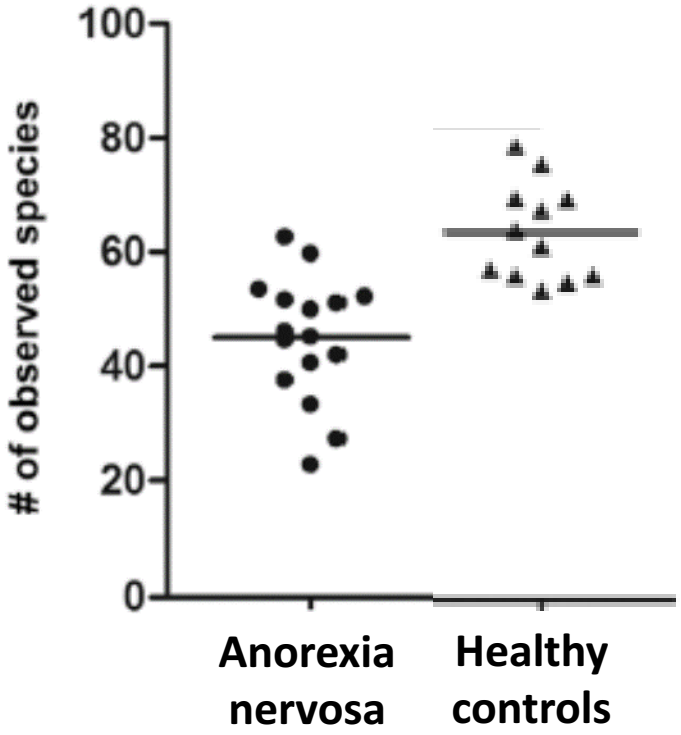
Monira.. Alam. *Front Microbiol* 2011;2:228.

ANOREXIA NERVOSA PATIENTS ALSO HAVE "DYSBIOSIS" WITH DECREASED DIVERSITY





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

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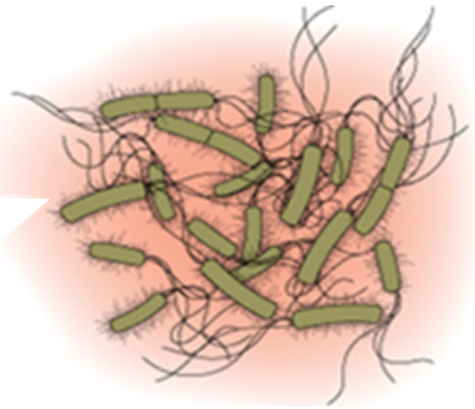
Borgo.. Borghi. *PLoS One* 2017; 12:e0179739.
Kleiman.. Carroll. *Psychosom Med* 2015;77:969-81.

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.

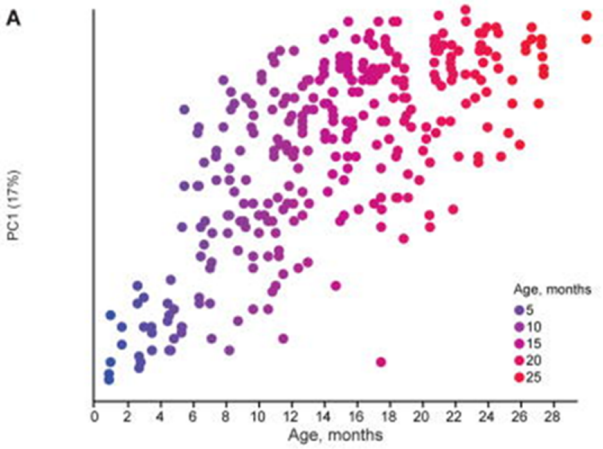


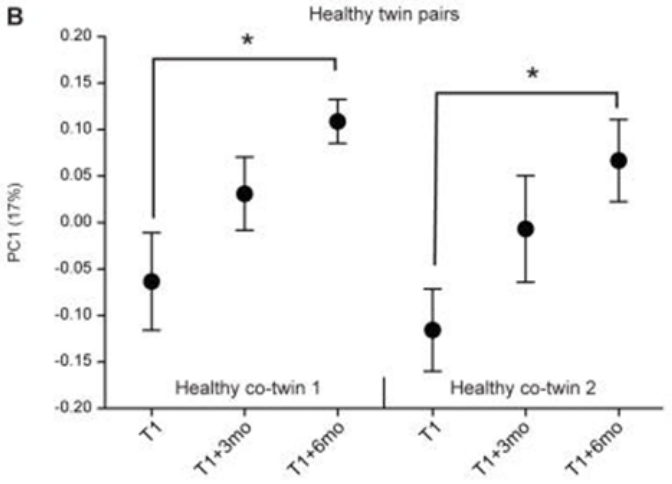
Microbial dysbiosis ±Enteropathogens

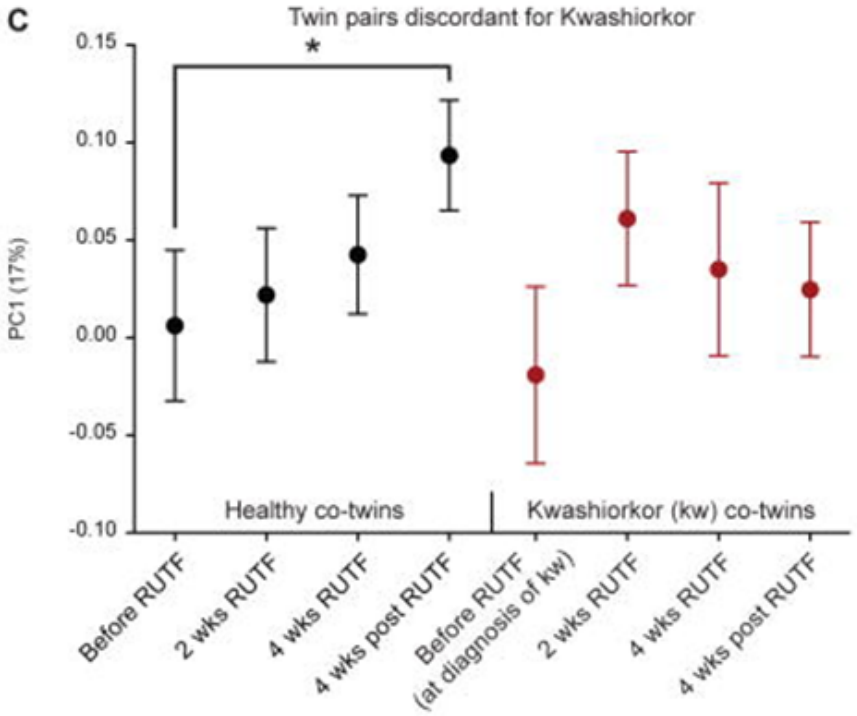
- **Decreased** abundance of genera with potentially **beneficial microbes**, including *Roseburia*, *Faecalibacterium*, *Butyrivibrio*, *Lactobacillus*, and *Bifidobacterium*.

Ghosh.. Nair. *PLoS One* 2014;9:1-13.
Gupta.. Mande. *Gut Pathog* 2011;3:1-7.
Monira.. Alam. *Front Microbiol* 2011;2:228.
Dinh.. Ward. *PLoS One* 2016;11:e0155405.

GUT MICROBIOTA MATURITY IS IMPAIRED IN CHILD UNDERNUTRITION







Smith.. Gordon. *Science* 2013;339:548-54.

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

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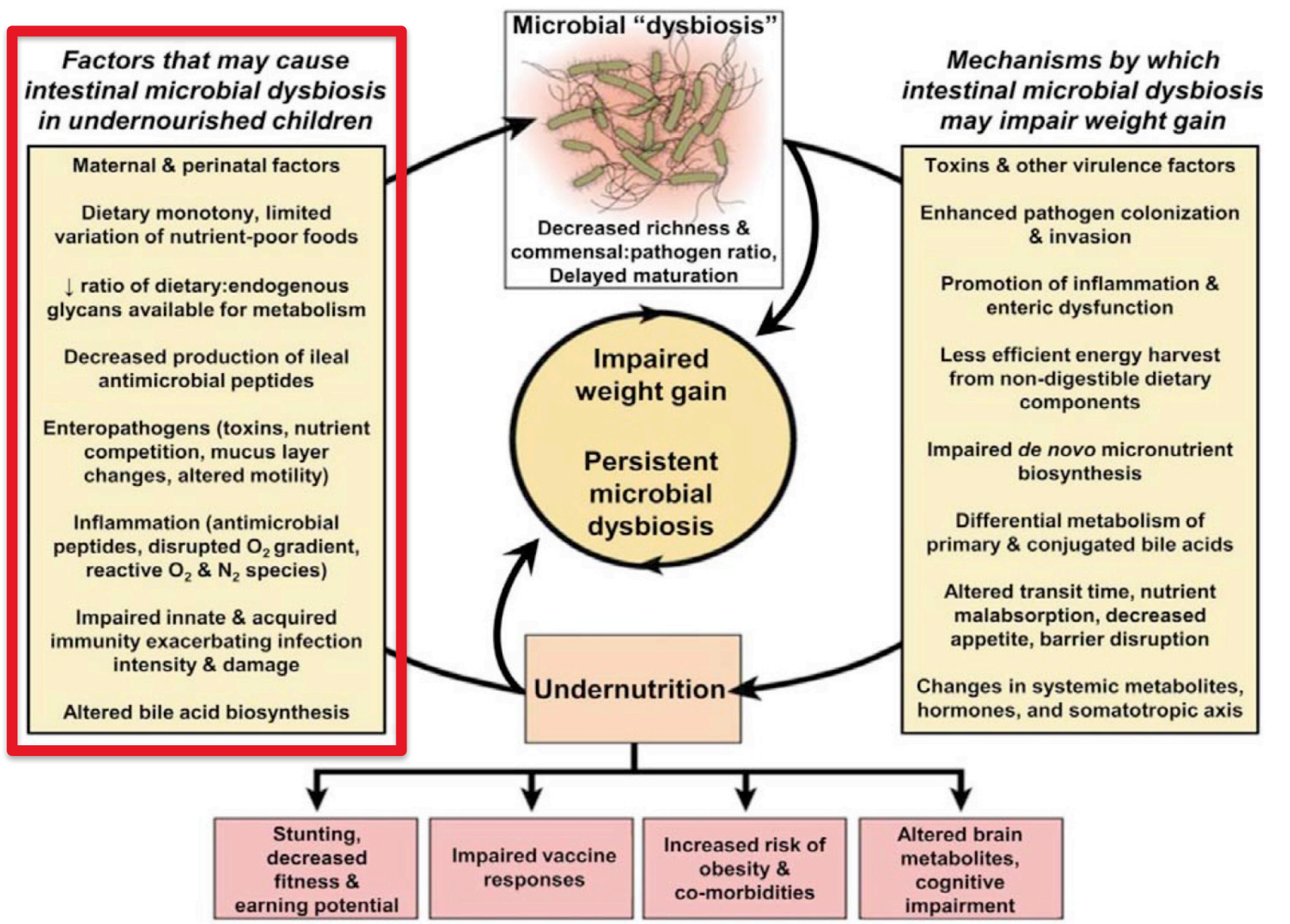

Velly.. Preidis. *Gut Microbes* 2017;8:98-112.

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

   Morris & Schmidt. *Nat Rev Microbiol* 2013;11:205-12.

   Albenberg.. Wu. *Gastroenterol* 2014;147:1055-63.

   Marteyn.. Tang. *Nature* 2010;465:355-61.

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

   Winter.. Baumler. *Nature* 2010;467:426-9.

   Winter.. Baumler. *Science* 2013;339:708-11.

DIET (CARBOHYDRATE CONTENT) INFLUENCES A CHILD'S MICROBIOME

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.

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De Filippo.. Lionetti. *Proc Natl Acad Sci USA* 2010;107:14691-6.

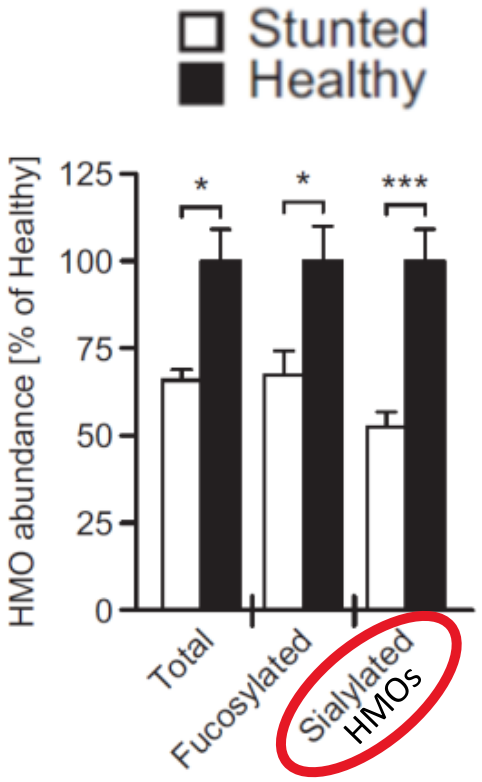
DIET (BREAST MILK VS FORMULA) INFLUENCES AN INFANT'S MICROBIOME

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.

Backhed.. Wang. *Cell Host Microbe* 2015;17:690-703.

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?



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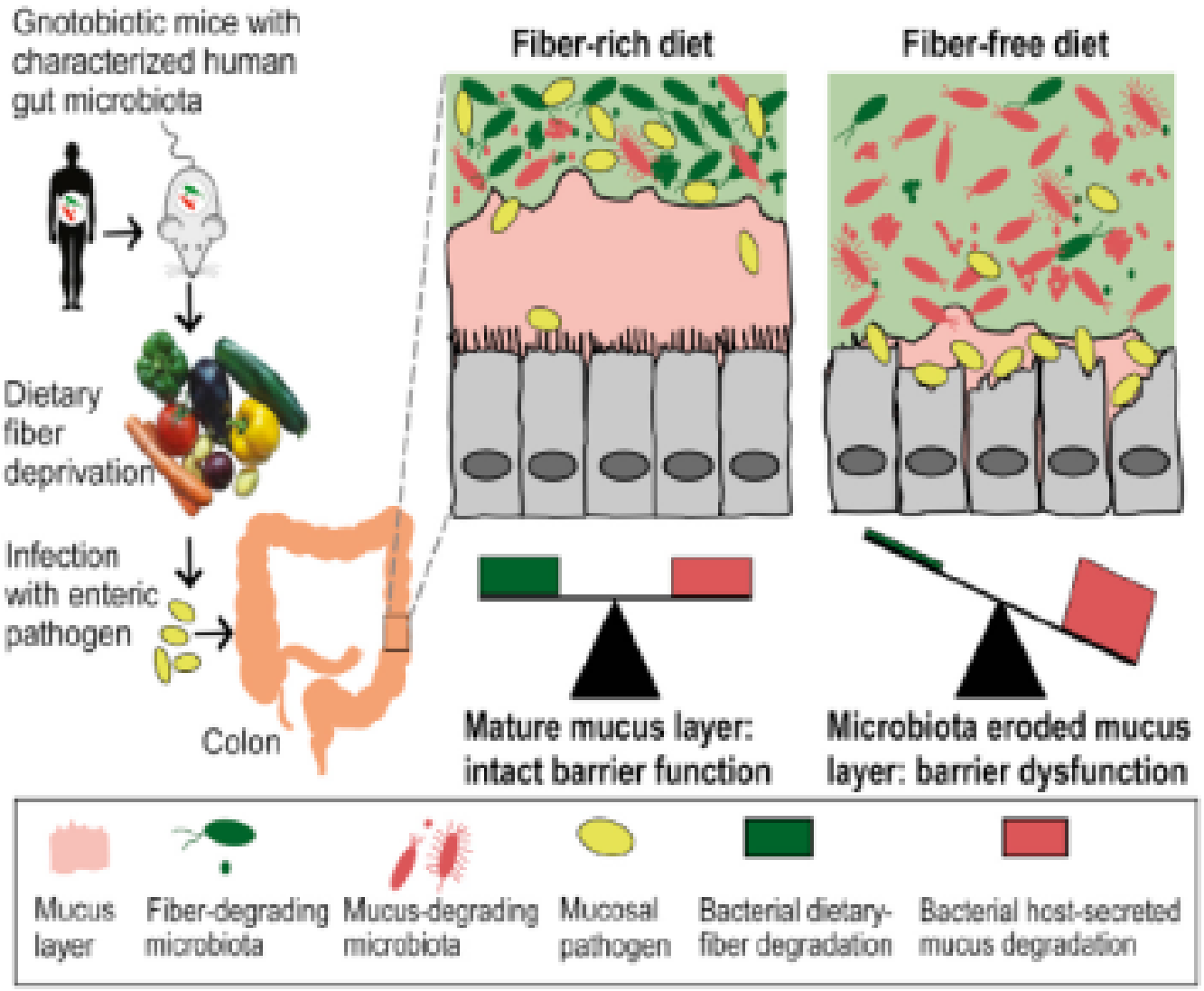
Charbonneau.. Gordon. *Cell* 2016;164:859-71.

DIET INFLUENCES THE INFANT MICROBIOME

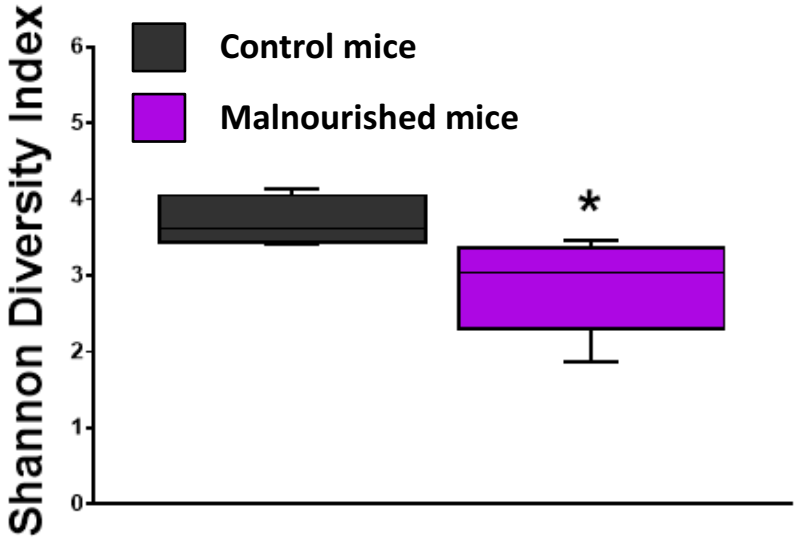
Charbonneau.. Gordon. *Cell* 2016;164:859-71.

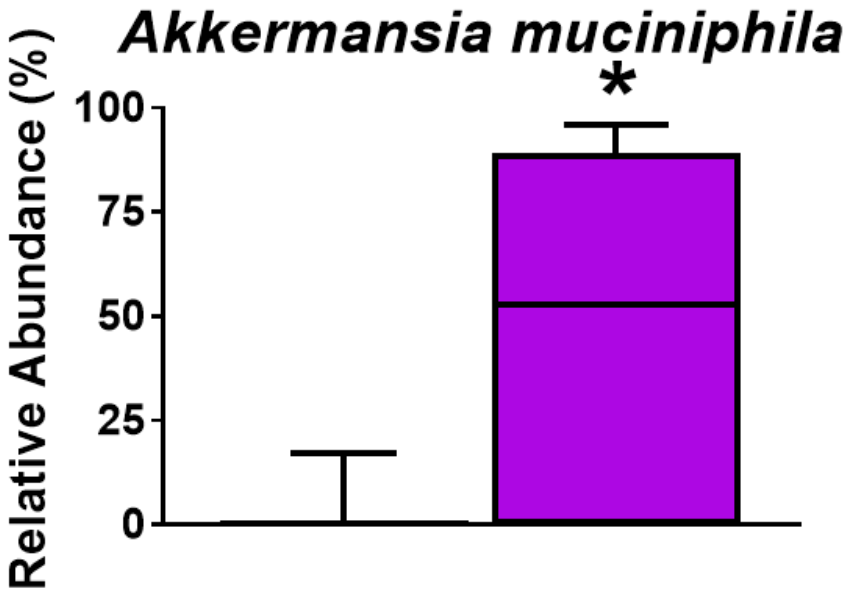
DIET-INDUCED GUT MICROBIAL "DYSBIOSIS" CAN CONFER SUSCEPTIBILITY TO INFECTION



Desai.. Martens. *Cell* 2016;167:1339-53.

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).

Santacruz.. Sanz. *Br J Nutr* 2010;104:83-92.

Karlsson.. Thorngren-Jerneck. *Obesity* 2012;20:2257-61.

Preidis.. Petrosino. *J Nutr Biochem* 2015;26:1050-7.

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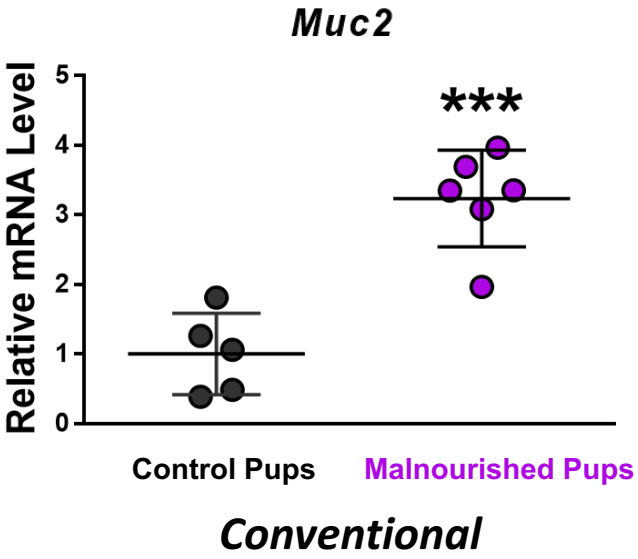
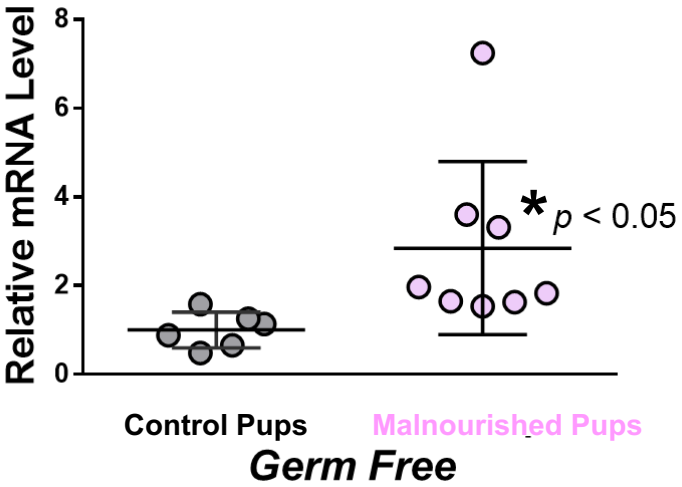
THIS OVERABUNDANCE OF MUCOLYTIC MICROBES IS ASSOCIATED WITH INCREASED HOST MUCIN GENE EXPRESSION





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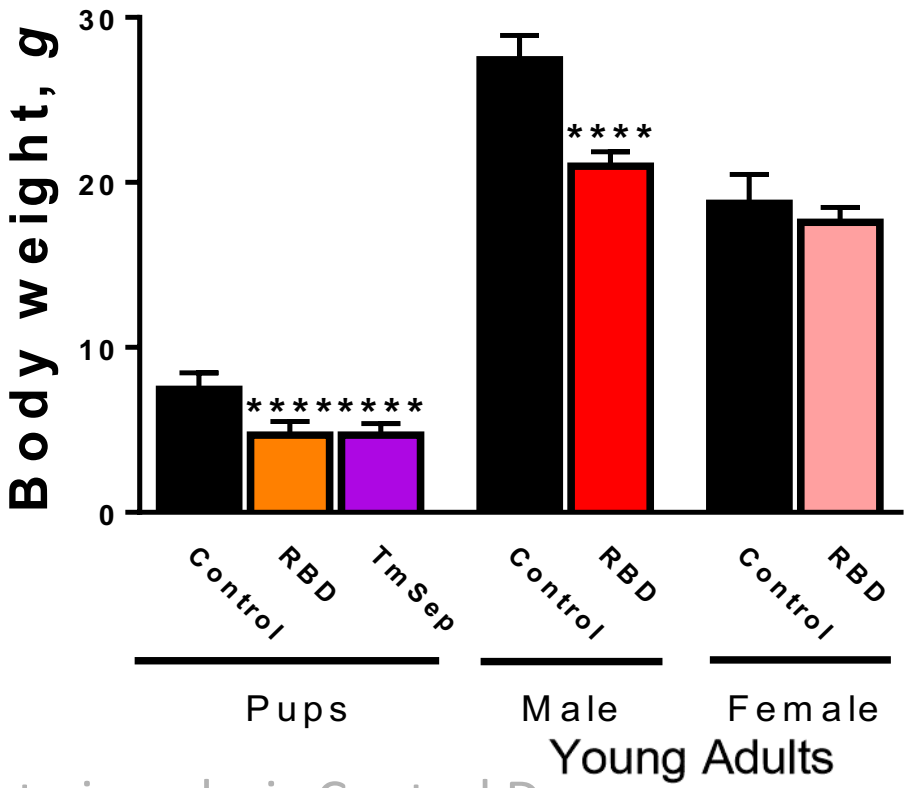
THREE MOUSE MODELS OF EARLY-LIFE UNDERNUTRITION

- **Timed Separation (TmSep)**
  - TmSep: 12 hours/day away fr
  - Controls: litters of normal pu
- **Regional Basic Diet (RBD) F**
  - RBD: Mothers fed 5% fat, 7%
  - Controls: Mothers fed isocal
- **RBD Young Adults**
  - RBD Pups weaned to RBD ch
  - Controls: Control Pups weaned to isocaloric Control Diet



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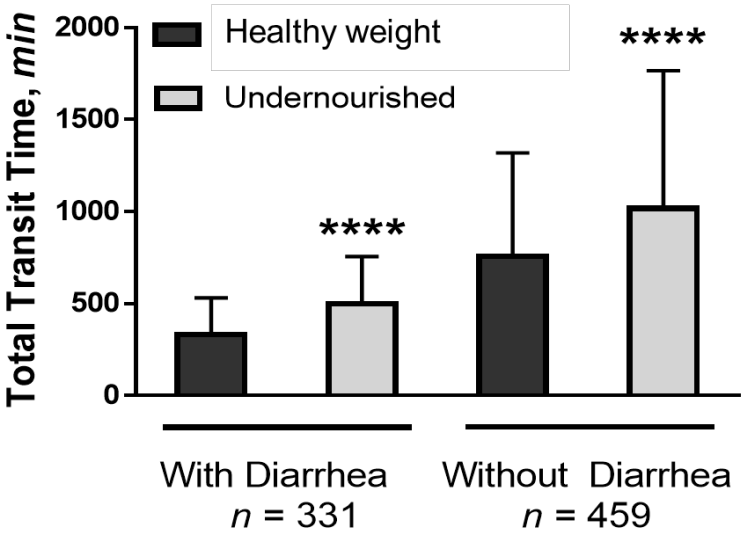
UNDERNOURISHED CHILDREN HAVE SLOW GASTROINTESTINAL MOTILITY



Viteri & Schneider. *Med Clin North Am* 1974;58: 1487-505.

Slow transit in undernutrition is linked to bacterial overgrowth, abdominal distention, constipation, and blunted appetite.

GI transit time influences microbiome composition and function.

Reviewed in: Preidis.. Shulman. "The Microbiome in Neurogastroenterology." In: *Pediatric Neurogastroenterology.* C. Faure *et al.* (Eds.), 2017.

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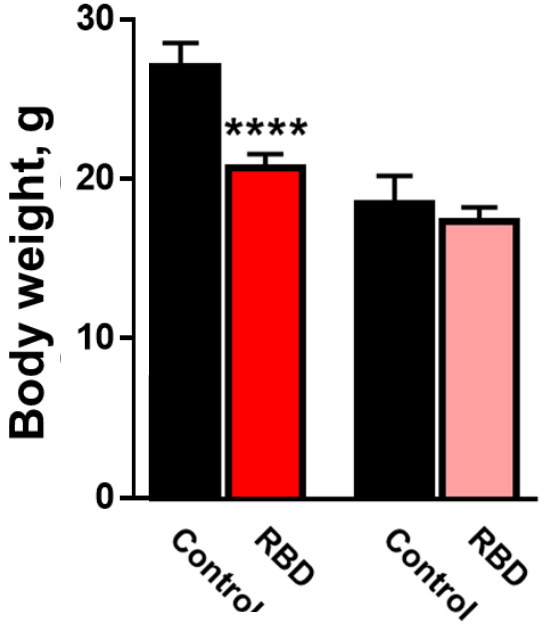
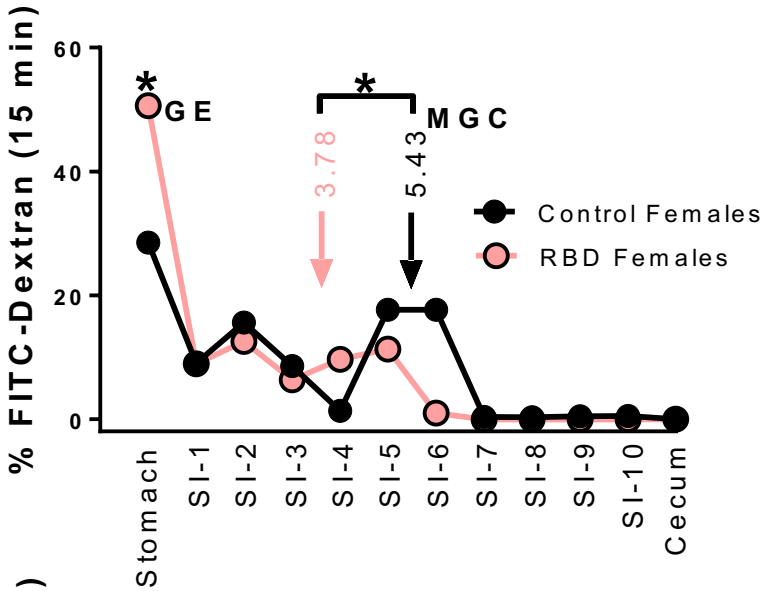
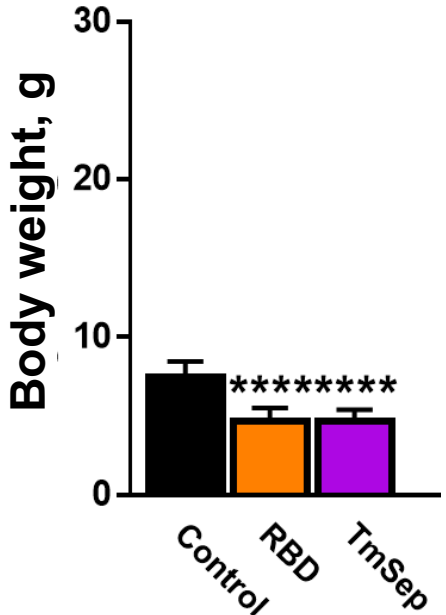
UNDERNUTRITION SLOWS GASTRIC AND SMALL BOWEL TRANSIT IN MULTIPLE MOUSE MODELS

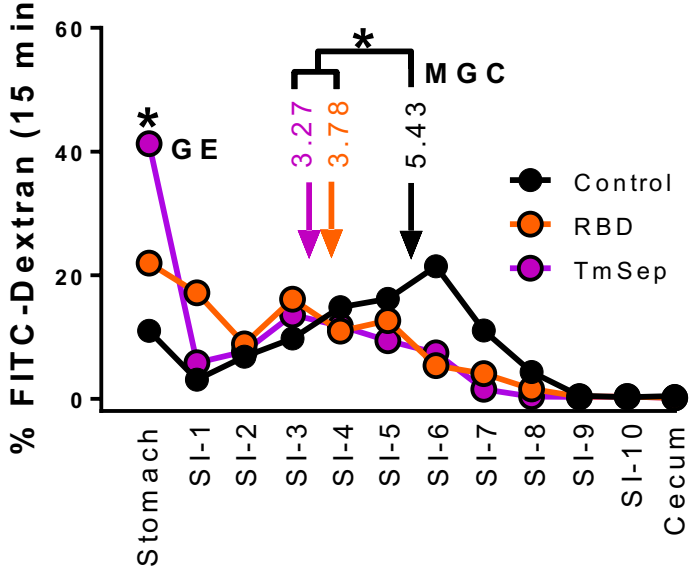


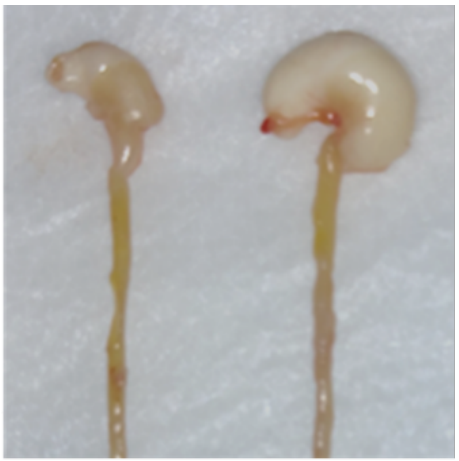


GE = gastric emptying
MGC = mean geometric center of bolused dye

Transit time in undernourished young adult <u>males</u> was minimally affected.







Control **TmSep**

UNDERNOURISHED CHILDREN HAVE DECREASED INTESTINAL BILE ACIDS

Luminal events of lipid absorption in protein-calorie malnourished children; relationship with nutritional recovery and diarrhea. II. Alterations in bile acid content of duodenal aspirates[1]

Roberto E. Schneider,[2] M.D. and Fernando E. Viteri,[3] M.D., D.Sc.

ABSTRACT Duodenal aspirates from PCM patients and from children with diarrhea present quantitative and qualitative alterations in their content of bile acids, consisting mainly in decreased concentrations of conjugated bile acids (CBA) and increased amounts of primary and secondary free bile acids (FBA). In the absence of diarrhea, PCM was associated with relatively uniform decrements of CBA, increasing to normal levels with nutritional recovery; the amounts of primary and secondary FBA did not change significantly with recovery. The effect of diarrhea on the bile composition of the children varied depending on the patient's nutritional status: *a*) In recovered children with diarrhea, all CBA, especially the taurine conjugates, were decreased; the glycine/taurine (G/T) ratios were above 1:4 in most of these patients. Although all FBA increased with diarrhea, such an increase was most significant in cholic and lithocholic acids; these children also presented high FBA/CBA ratios. *b*) In PCM children, the degree of decrement of CBA during diarrhea was similar in taurine and glycine conjugates, the G/T ratio remaining within normal limits. The concentrations of primary and secondary FBA did not change significantly in PCM children with diarrhea when compared with the group without diarrhea. It is believed that the changes in bile acids observed in these children are due to the interaction of malnutrition, diarrhea, and an increased gastrointestinal flora. A theory of how the bile acid events described herein occur is also proposed. *Am. J. Clin. Nutr.* 27: 788–796, 1974.

Schneider & Viteri. *Am J Clin Nutr* 1974;27: 788-96.

Decreased bile acids in undernutrition are linked to dietary fat malabsorption, fat-soluble vitamin deficiencies, bacterial overgrowth, and poor weight gain during refeeding.

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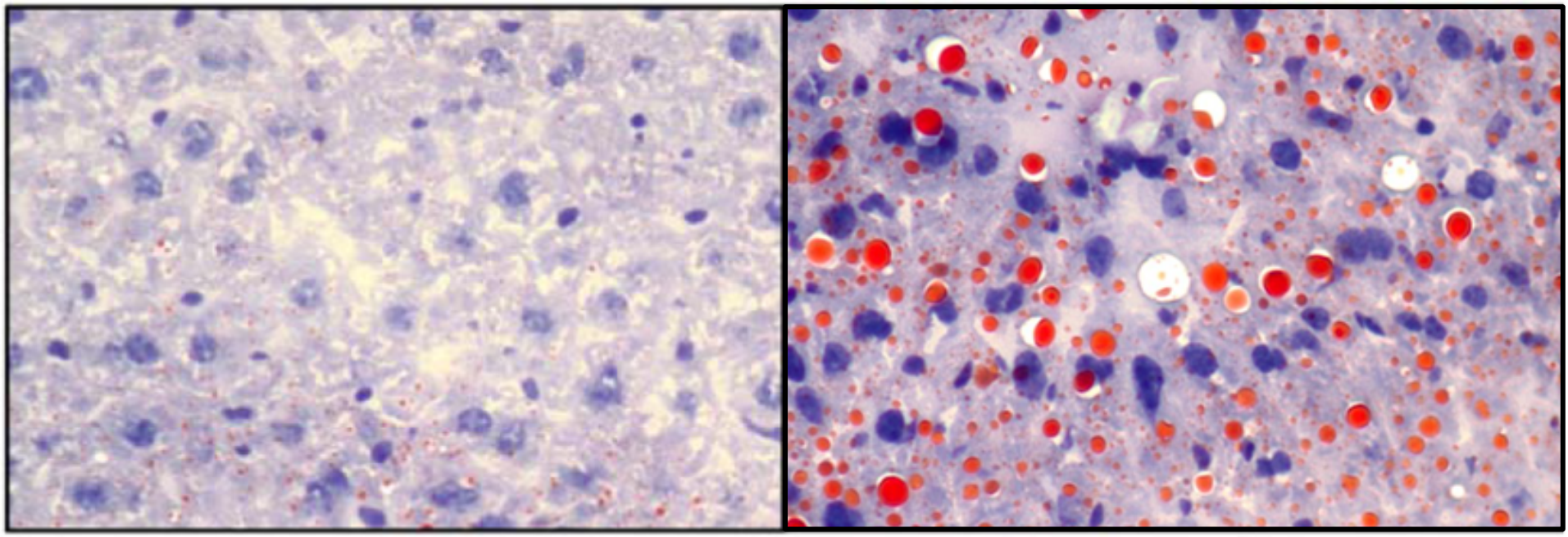
Intestinal bile acids help regulate gut microbial populations.

Reviewed in: Jia.. Jia. *Nat Rev Gastroenterol Hepatol* 2017; in press.

UNDERNOURISHED MICE (AND CHILDREN) EXHIBIT MACROVESICULAR STEATOSIS



Control **RBD Young Adults**

Oil red O stain, flash-frozen livers, x160

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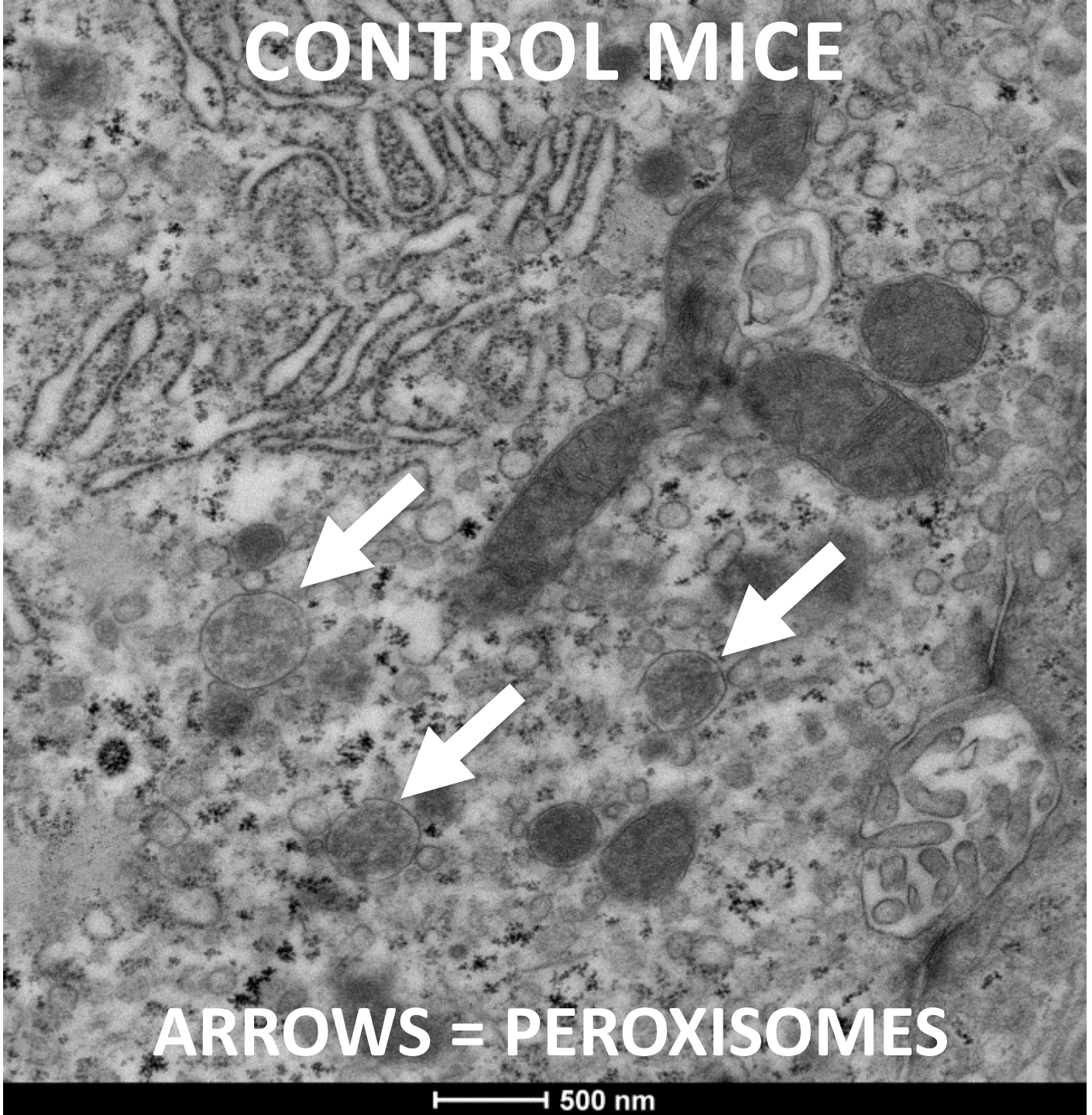
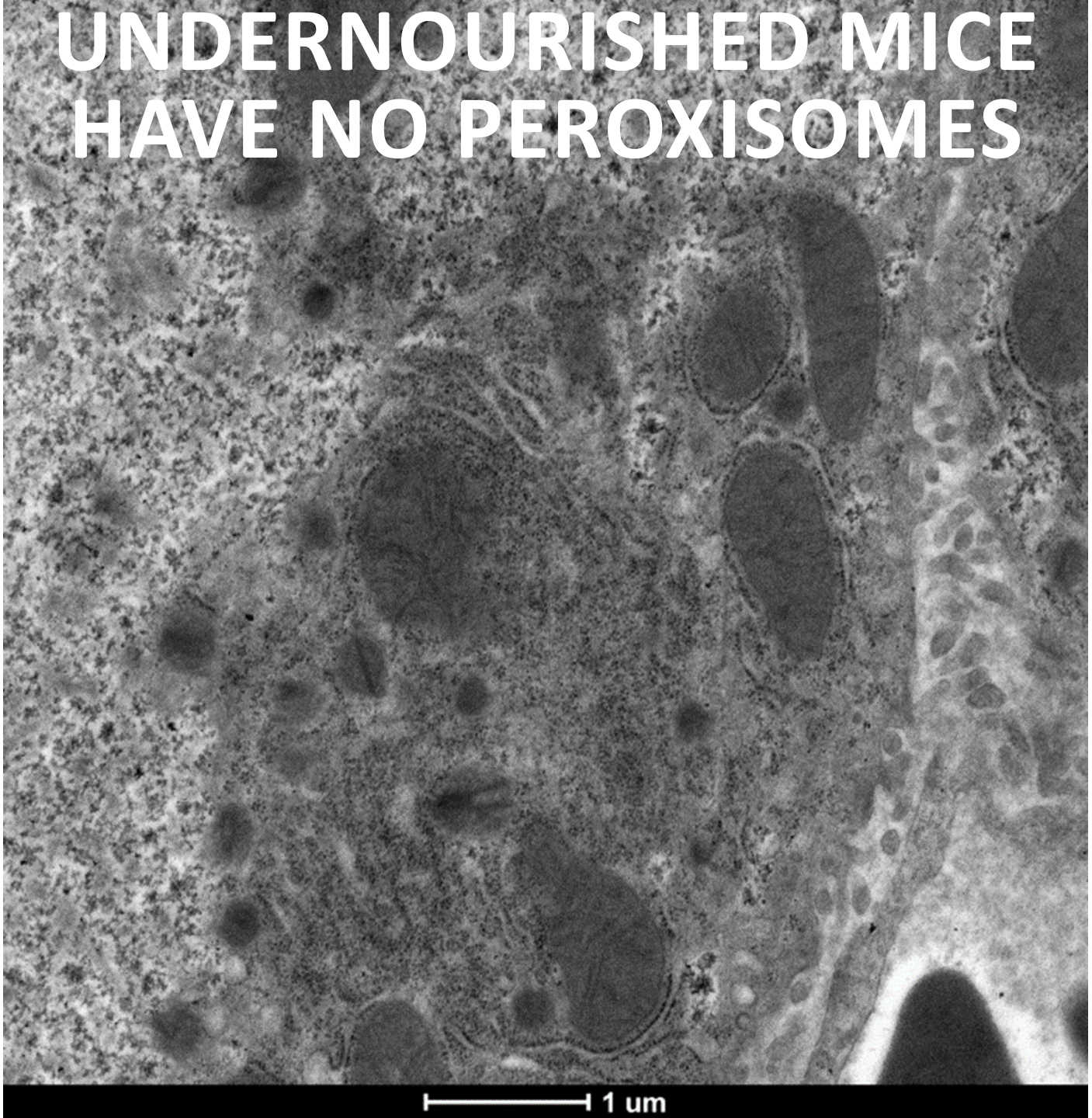
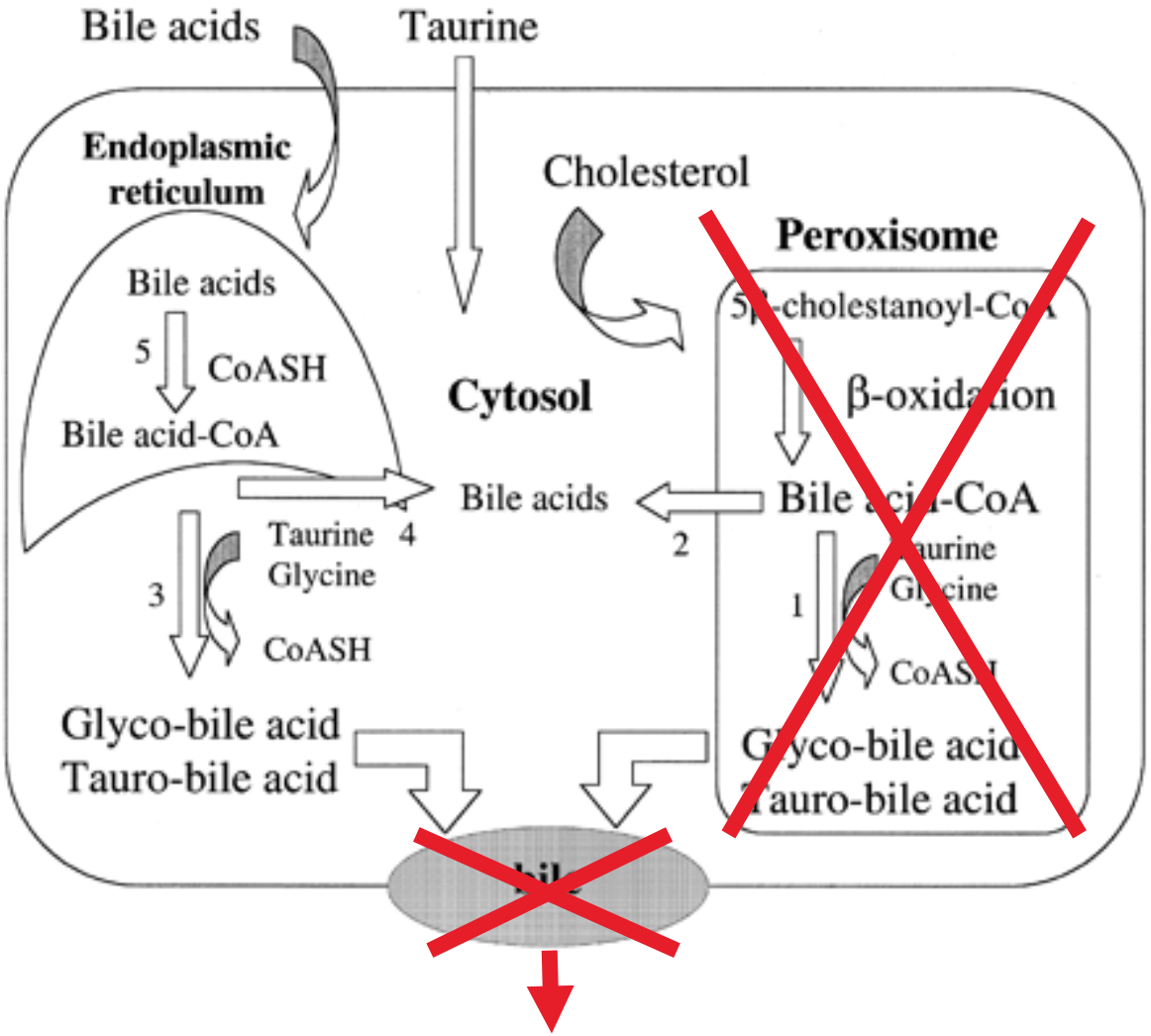

CONTROL MICE
ARROWS = PEROXISOMES
500 nm


UNDERNOURISHED MICE
HAVE NO PEROXISOMES
1 um



Altered Intestinal Microbiota?

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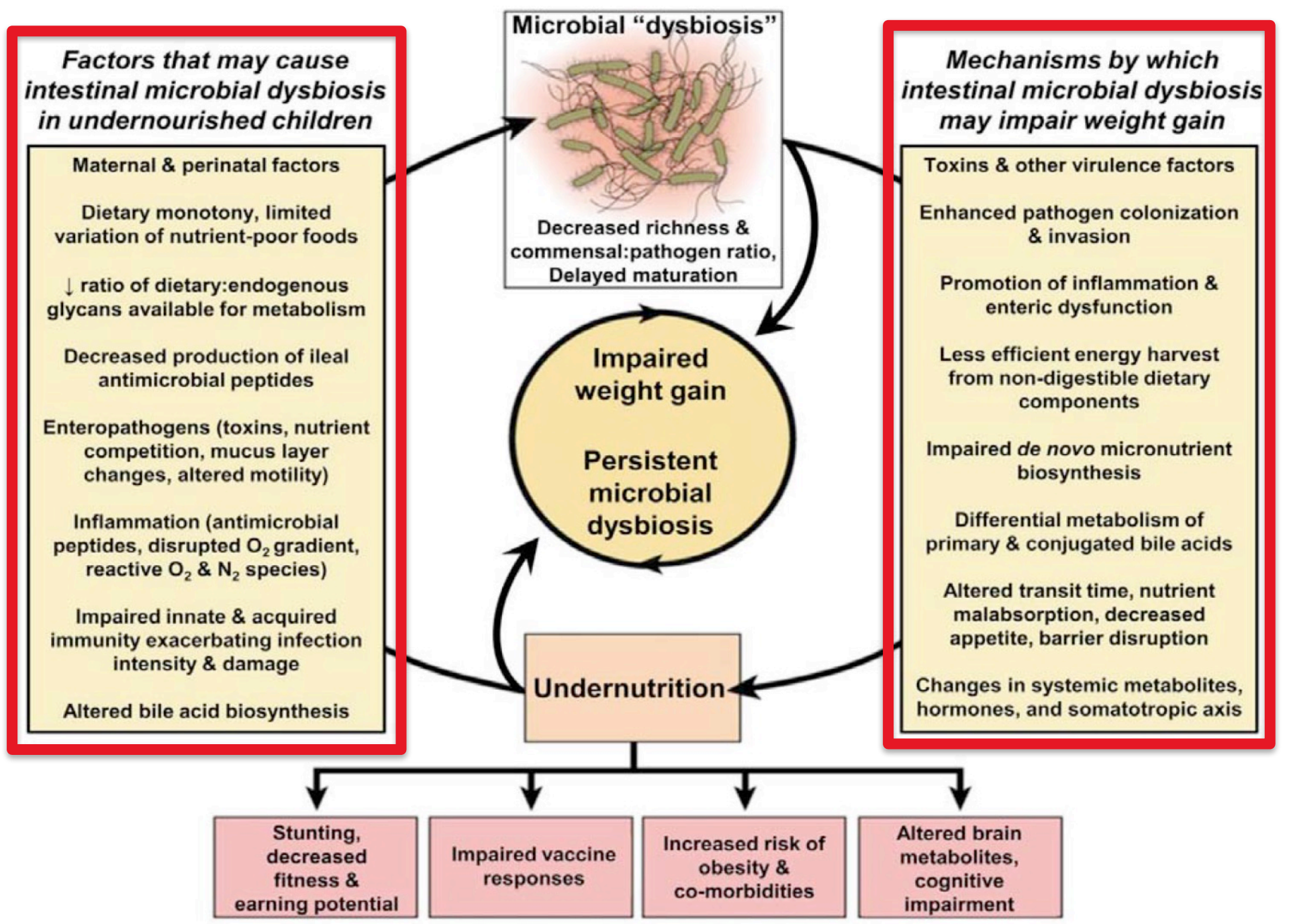
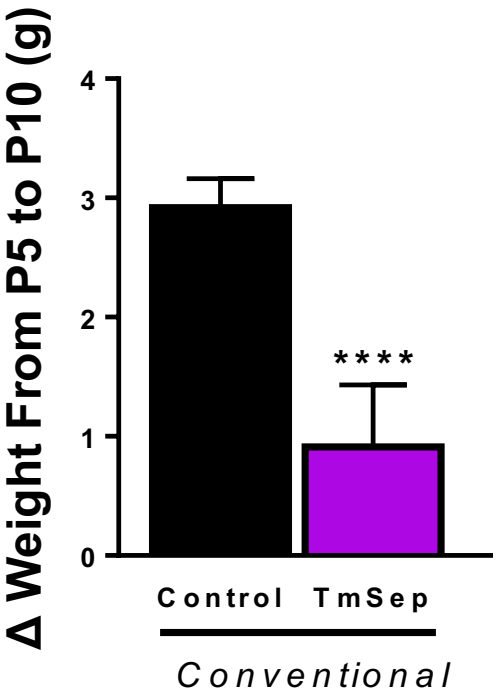
Solaas.. Kase. *J Lipid Res* 2000;41:1154-62.



Velly.. Preidis. *Gut Microbes* 2017;8:98-112.

GERM-FREE MICE TOLERATE EARLY-LIFE UNDERNUTRITION BETTER THAN CONVENTIONAL MICE WITH INTESTINAL BACTERIA



n = 10-18

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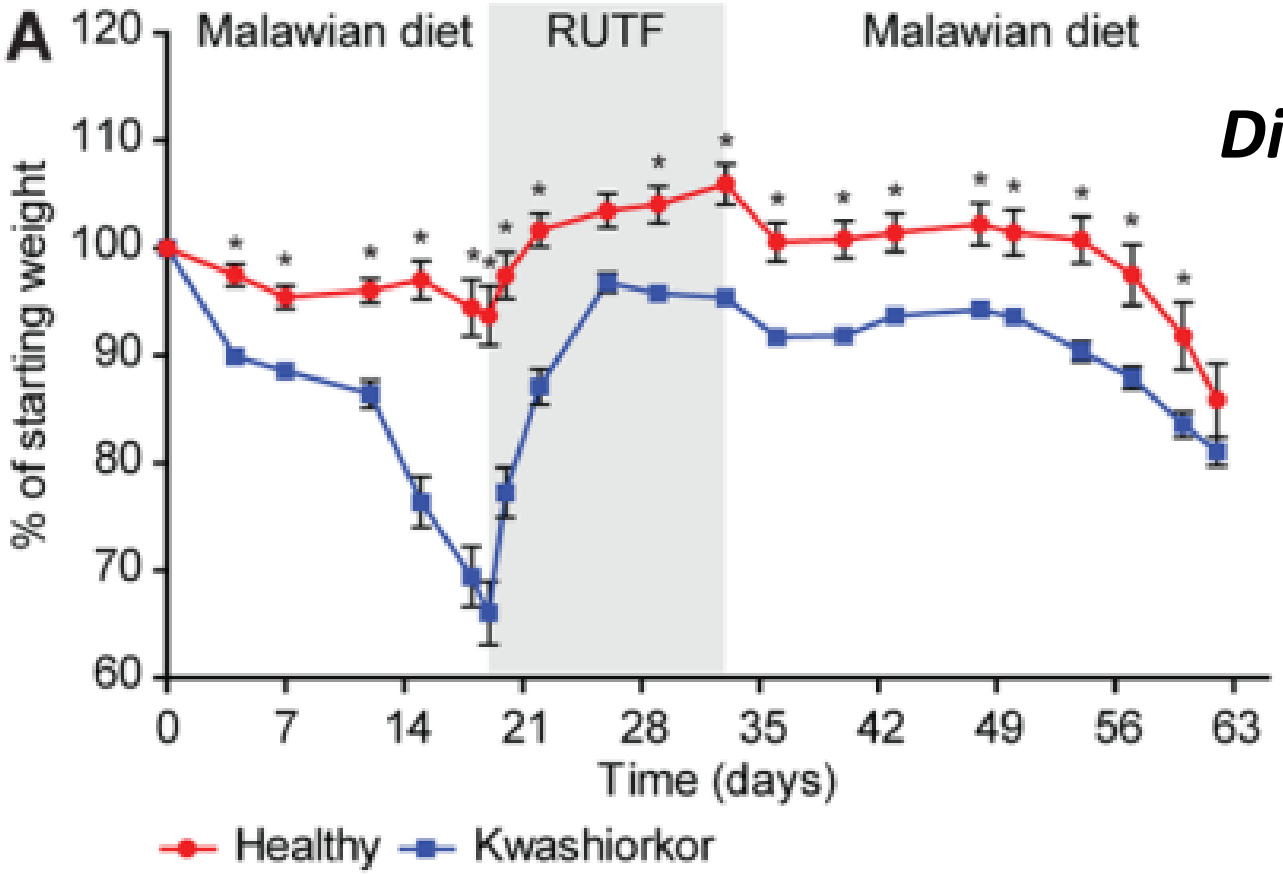
FECAL MICROBES FROM UNDERNOURISHED CHILDREN CAN CAUSE UNDERNUTRITION IN GNOTOBIOTIC MICE (UNDER THE RIGHT CONDITIONS)



Diet is key!

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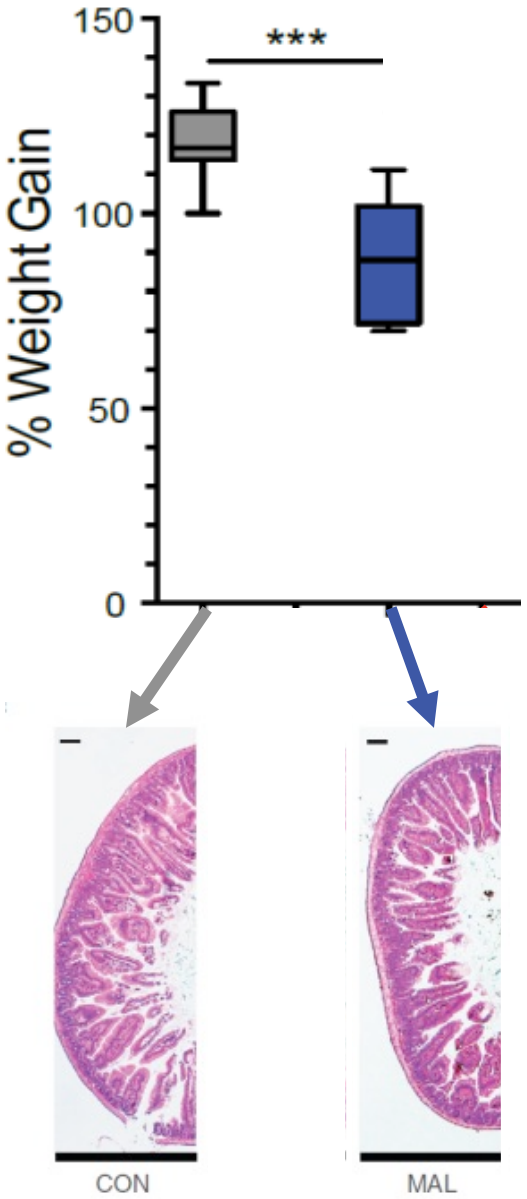
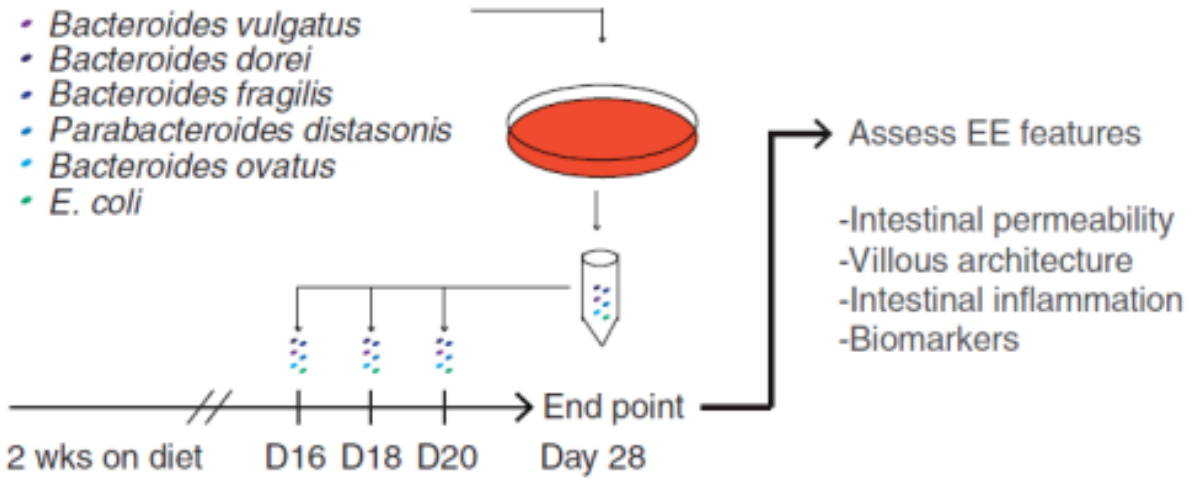
Smith.. Gordon. *Science* 2013;339:548-54.

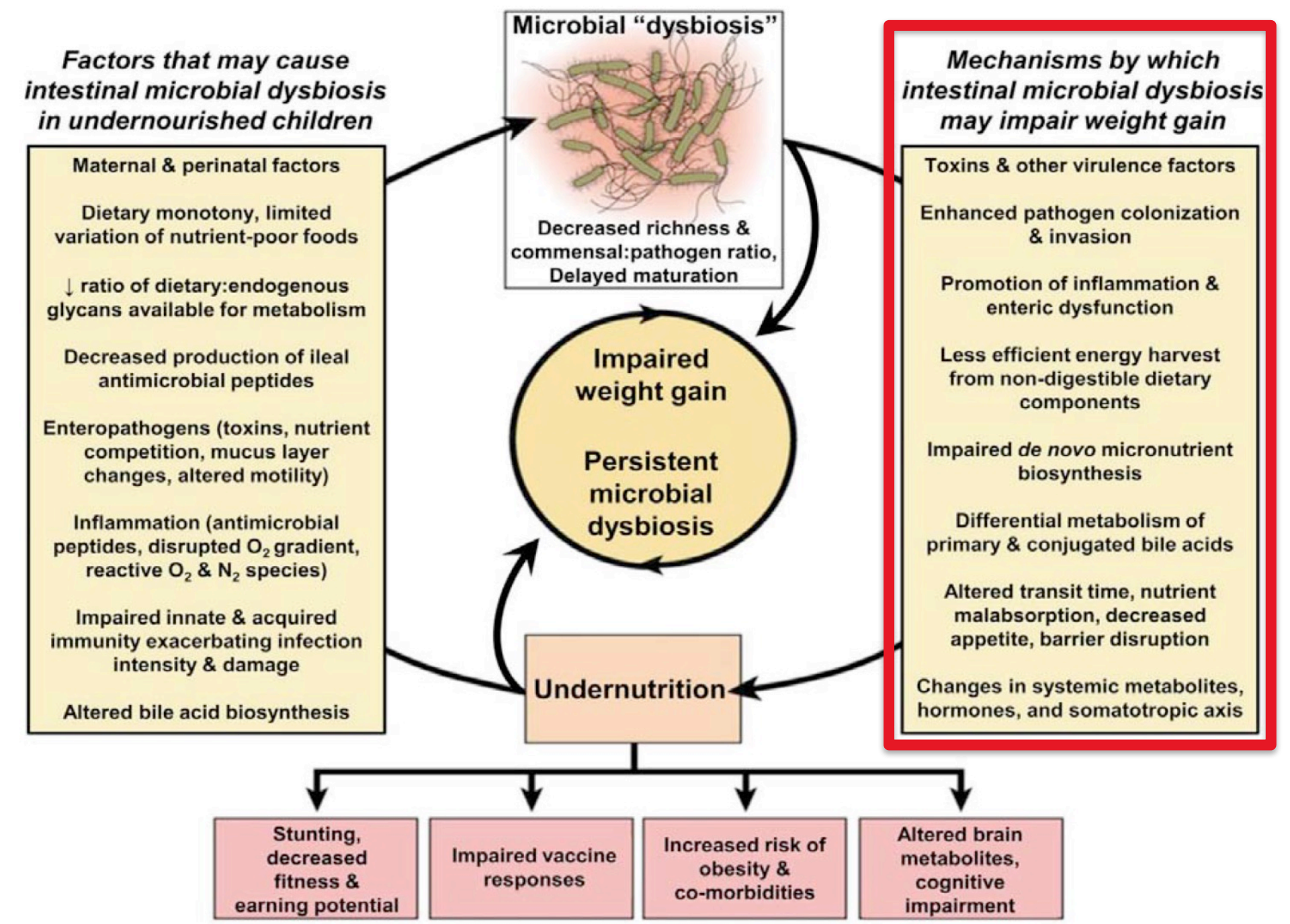
GUT BACTERIA (EVEN NON-PATHOGENS) CAN CAUSE INFLAMMATION AND GROWTH IMPAIRMENT



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



Brown.. Finlay. *Nat Comm* 2015;6:7806.



Velly..Preidis. *Gut Microbes* 2017;8:98-112.

KEY POINT 2

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

Trehan.. Manary. *N Engl J Med* 2013;368:425-35.

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**

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Isanaka.. Grais. *N Engl J Med* 2016;374:444-53.
Berkley.. Fegan. *Lancet Global Health* 2016;4:e464-73.

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

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Kerac.. Collins. *Lancet* 2009;374:136-44.

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

Kerac.. Collins. *Lancet* 2009;374:136-44.

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.

Intervention	Setting	Number of Study Participants	Result	Reference
Synbiotic 2000 Forte	Malawi	795	No significant effect on nutritional cure	104
Amoxicillin or cefdinir	Malawi	2767	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)	105
Amoxicillin	Niger	2412	No significant effect on nutritional recovery	106
Co-trimoxazole	Kenya	1778	No significant effect on mortality	107

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?

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Velly.. Preidis. *Gut Microbes* 2017;8:98-112.

KEY POINT 3

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

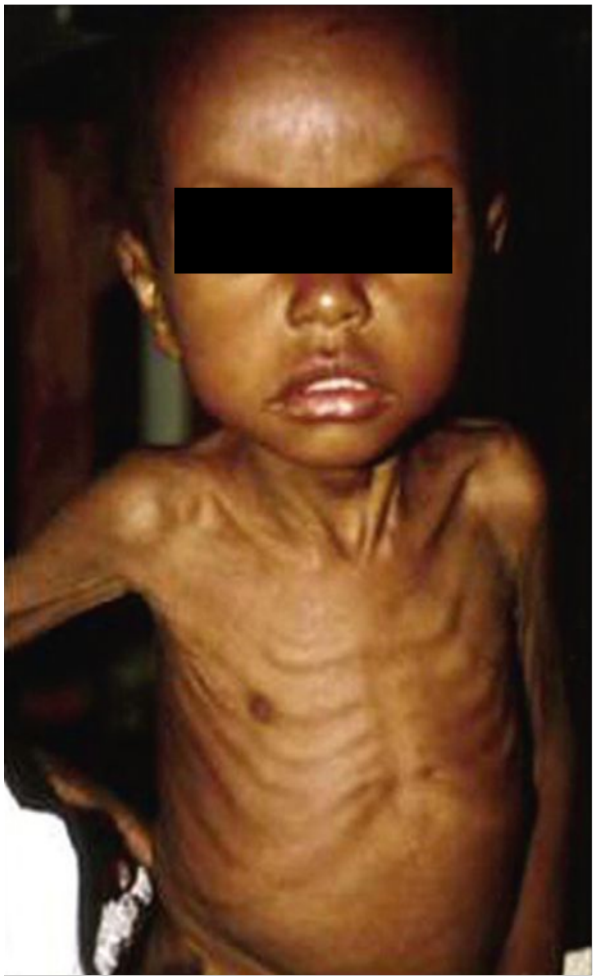
- Although there is currently not enough clinical evidence to recommend microbiome-targeting therapies for undernourished children, promising preclinical models suggest that individualized therapies might one day allow clinicians to improve a child's growth trajectory

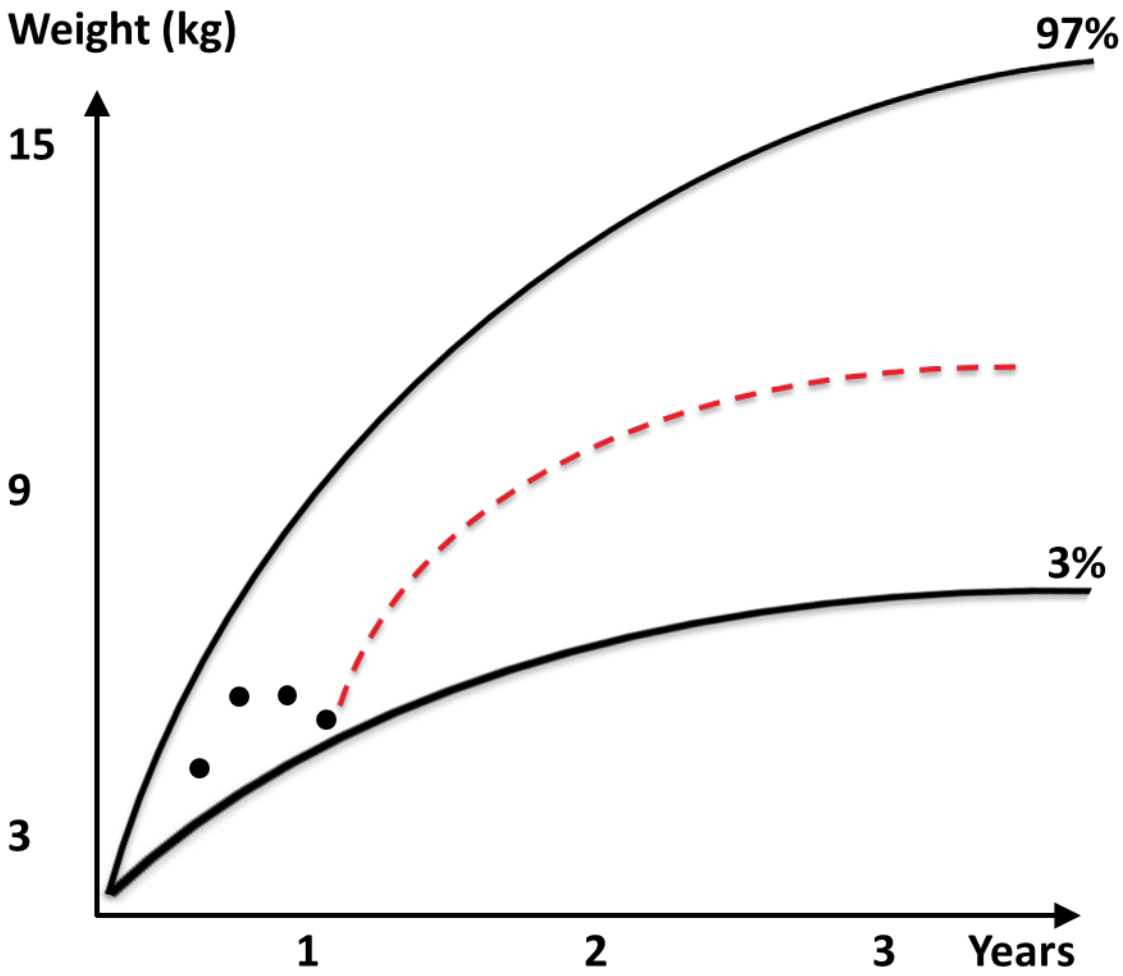
WHAT LIES AHEAD?

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





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