

Diet, child nutrition and the microbiome

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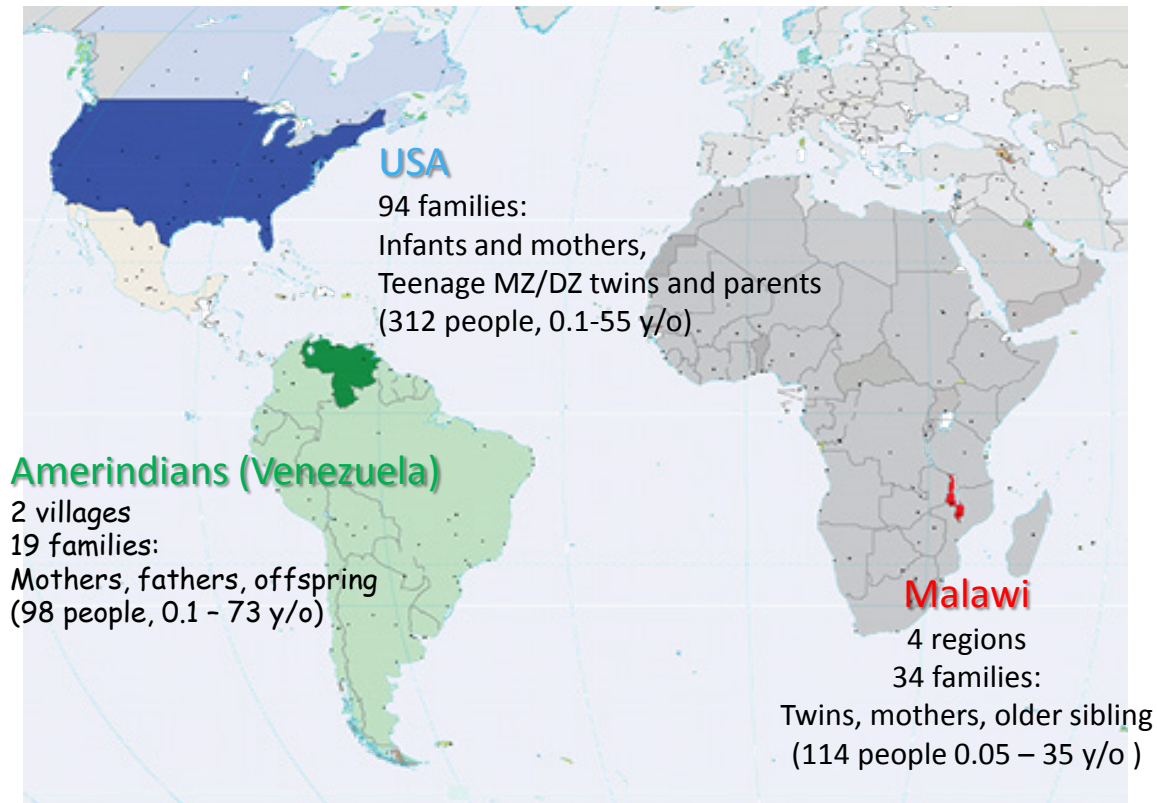
Key Questions

- How does diet in early life influence the microbiome?
- How does the microbiome influence child nutritional status?
- How do differences in microbiome structure and function affect nutritional outcomes during the first 2-3 years of life, as well as long-term health and developmental outcomes?

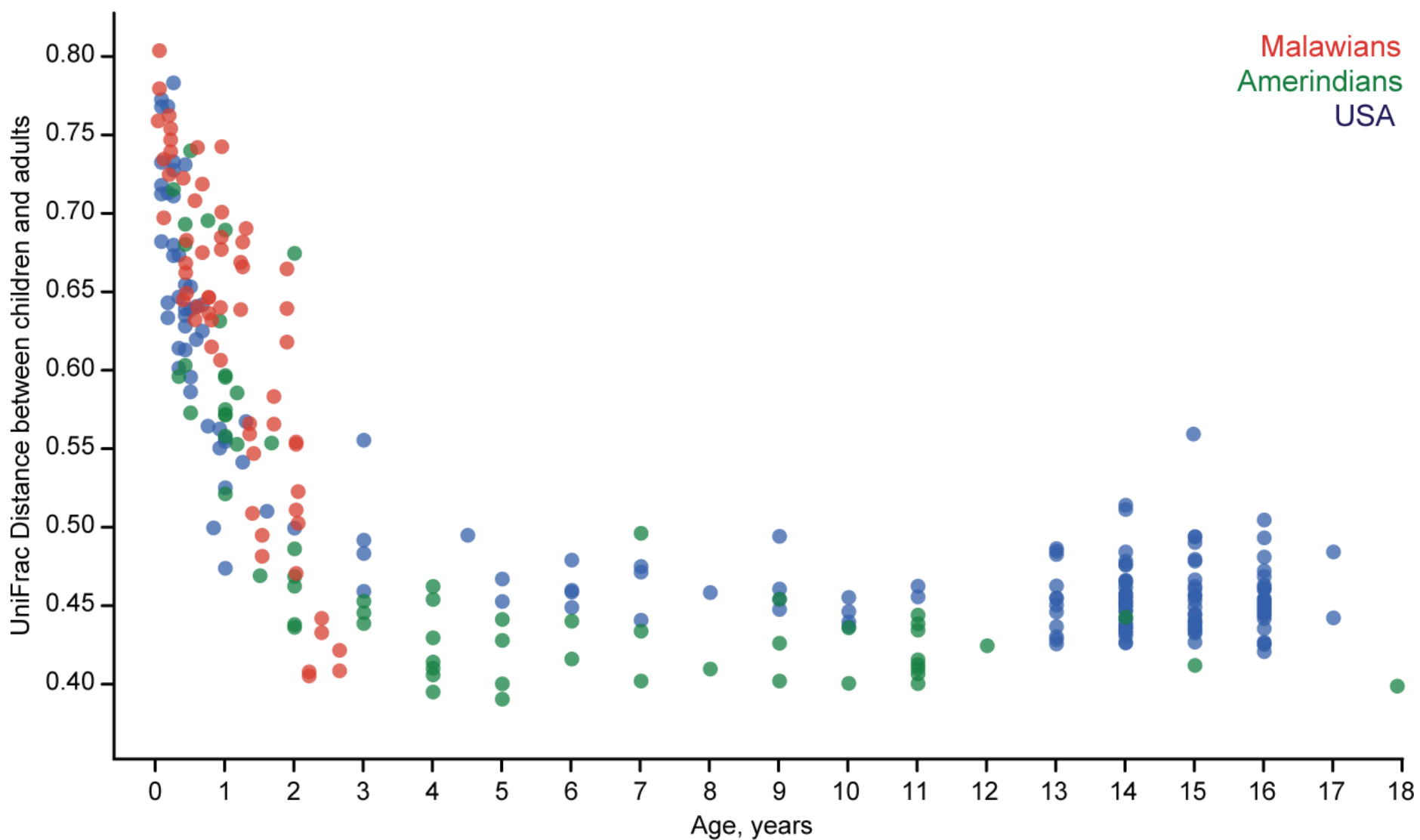
Infant microbiome

- Infant intestine initially a sterile environment
- Various factors determine individual gut microbiotic composition
 - Gestational age
 - Delivery mode: vaginal vs. C-section
 - Maternity ward/neonatal unit
 - Feeding mode: breast milk vs. formula
 - Other foods and fluids
 - Antibiotic use
 - Paternal skin
 - Environment

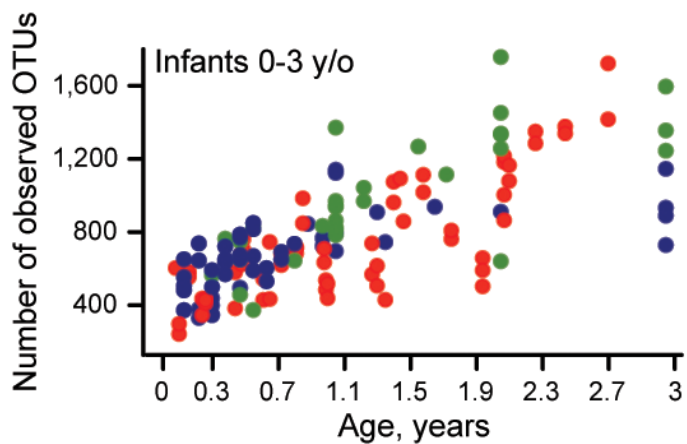
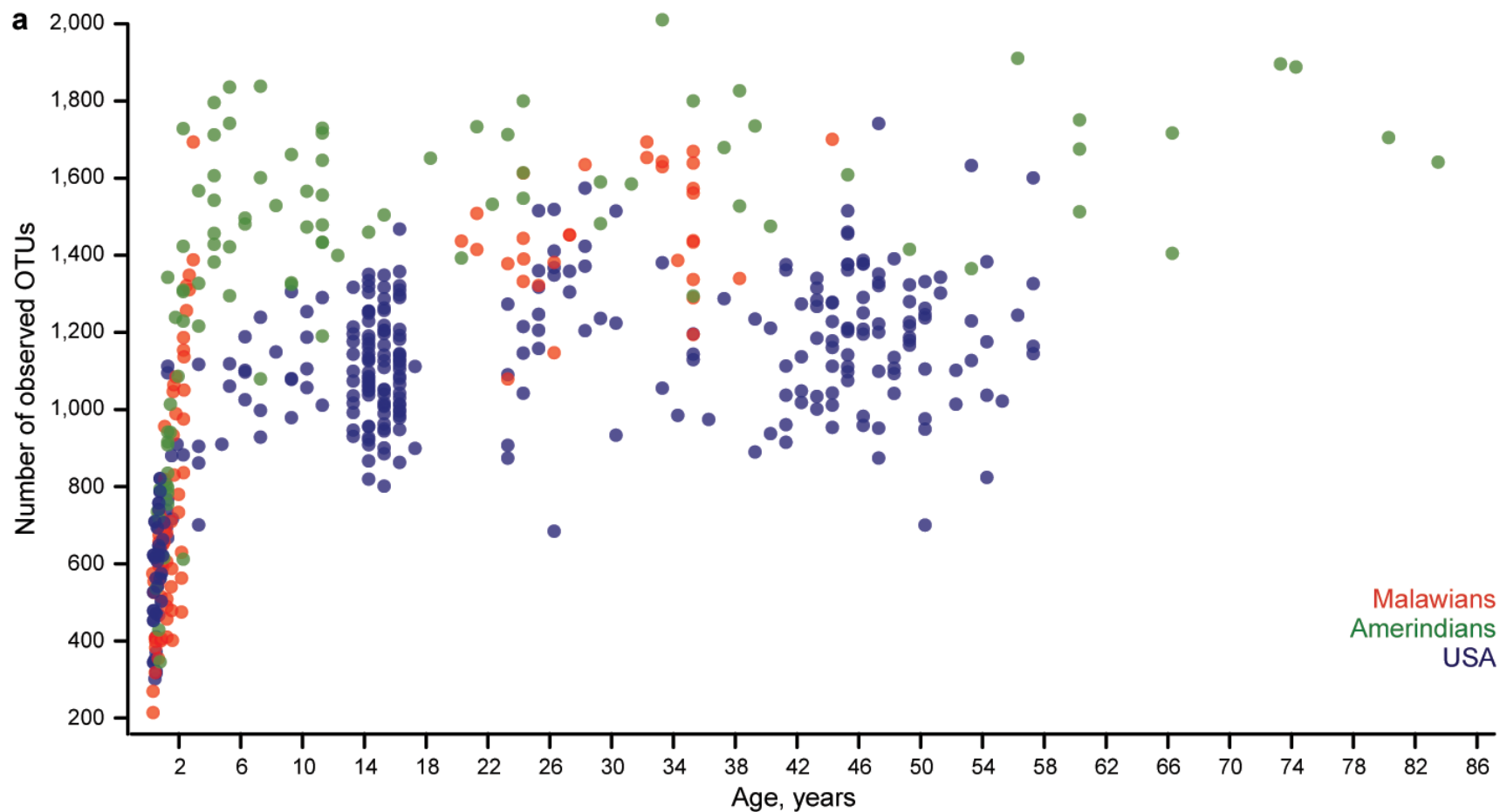
Human gut microbiome differentiation viewed across cultures, ages, and families



Characterized bacterial species present in fecal samples obtained from infants, children, teenagers and adults, parents and offspring, and included mono- and dizygotic twins



UniFrac distances between children and adults decrease with increasing age of children in each population. Each point shows an average distance between a child and all adults unrelated to that child but from the same country.



Bacterial diversity increases with age
in each population

The role of breastfeeding

- Microbes in breast milk “seed” the infant’s GI tract?
- Prebiotics in breastmilk (e.g. glycoconjugates) promote growth of certain bacteria e.g. Bifidobacteria
- Microbiome of breastfed vs. formula-fed infants differs

Microbes in breast milk

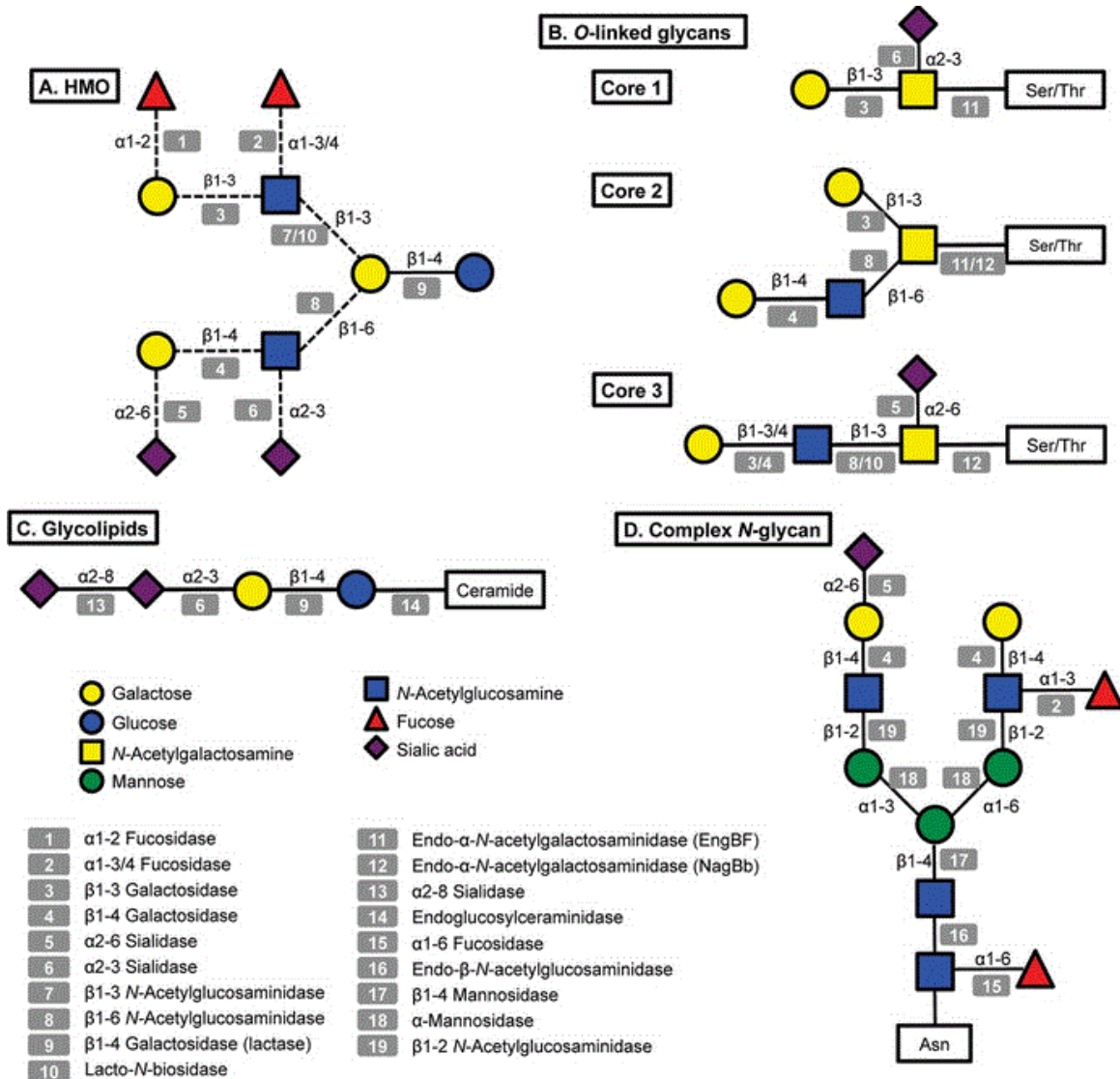
- Milk microbial community characterized for 18 lactating women within 2 d of childbirth and at 1 and 6 mo postpartum
- Several hundred species of bacteria likely – compositionally distinct from other human niches, not simply contaminants from skin
- Colostrum has higher microbial diversity than transitional and mature milk

Microbes in breast milk

- Weisella, Leuconostoc, Staphylococcus, Streptococcus, and Lactococcus were predominant in colostrum samples
- In milk samples at 1 and 6 mo, the typical inhabitants of the oral cavity (e.g., Veillonella, Leptotrichia, and Prevotella) increased (bacteria from infant's mouth colonize milk ducts and areola?)
- Milk from obese mothers tended to contain a different and less diverse bacterial community compared to milk from normal weight mothers
- Milk samples from mothers with elective (but not non-elective) C-section contained a different bacterial community than milk samples from mothers with vaginal delivery

Prebiotics in human milk

Consumption of human milk glycoconjugates by infant-associated bifidobacteria: mechanisms and implications [Garrido et al. 2013]



Structural diversity of glycans in human milk and corresponding glycosyl hydrolases in infant-gut associated bifidobacteria. Legends at the bottom left indicate monosaccharide composition and the corresponding potential glycolytic enzymes in bifidobacteria acting at specific linkages. A: illustrative structure of HMO; B: three different cores found in human O-linked glycans; C: glycolipids, the structure of ganglioside GD3 is shown; D: a complex N-glycan.

Microbiome of breastfed vs. formula-fed infants

Results are mixed, e.g.:

- Bifidobacteria dominated the microbiota of breastfed infants, whereas formula-fed infants had higher proportions of Bacteroides and members of the Clostridium coccoides and Lactobacillus groups (n=606; Fallani et al., 2010)
- Formula fed infants had increased richness of species, with overrepresentation of *Clostridium difficile*, but no difference in *Bifidobacteria* compared to breastfed infants (n=24; Azad et al., 2013)

Table 3 Results of studies comparing the gut microbiota of breastfed and formula-fed infants

Bacterial group	Number of studies					
	↑ In breastfed		No clear difference		↓ In breastfed	
	Before 1980	After 1980	Before 1980	After 1980	Before 1980	After 1980
Bifidobacteria	5/6	7/27	1/6	20/27	0/6	0/27
<i>Bacteroides</i>	0/3	2/19	1/3	9/19	2/3	8/19
Clostridia	0/3	0/17	0/3	4/17	3/3	13/17
Lactobacilli	NT*	2/16	NT	10/16	NT	4/16
<i>Enterobacteriaceae</i>	0/7	1/21	5/7	9/21	2/7	11/21
Enterococci	0/2	0/16	0/2	5/16	2/2	11/16
Staphylococci	NT	5/12	NT	7/12	NT	0/12

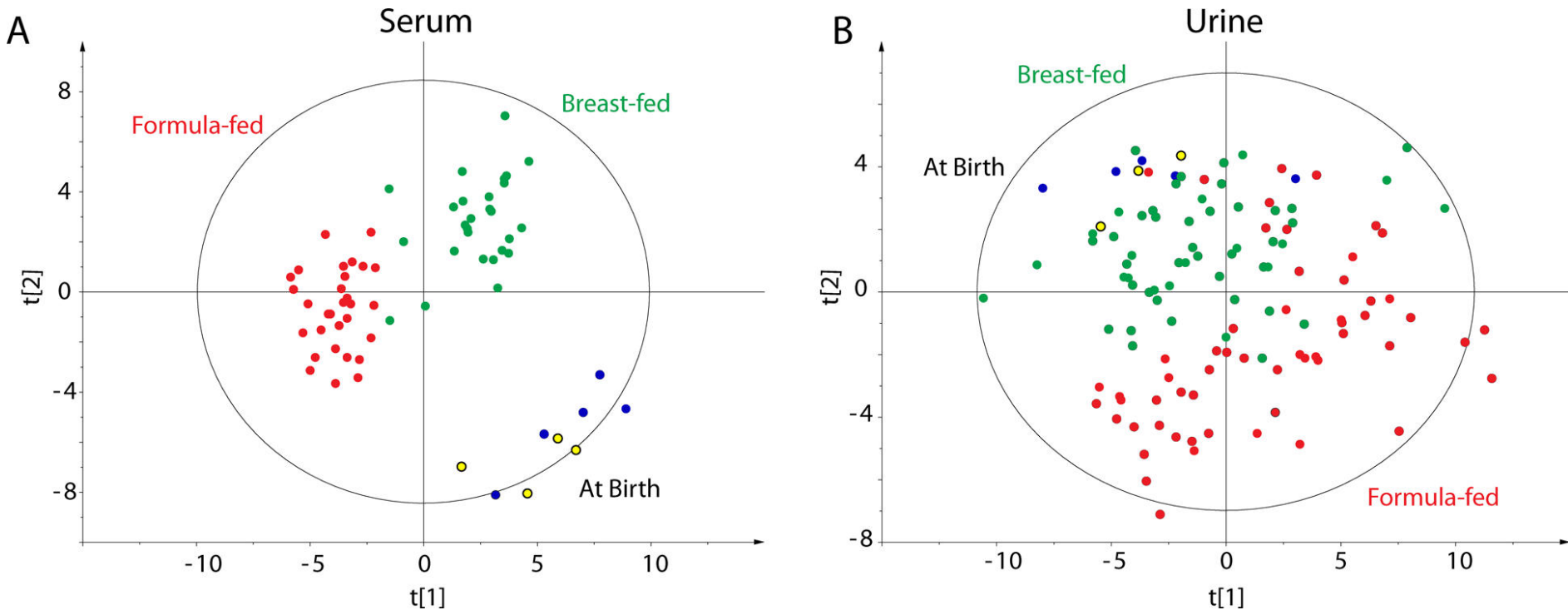
* not tested.

Data are summarised from 34 studies, including (21, 24, 25, 29, 34). A complete list of references can be obtained from the corresponding author.

Rhesus monkey model to compare breastfed vs. formula-fed infants

[O'Sullivan et al. J Proteome Res 2013]

- Rhesus monkey infants randomized to receive breast milk or formula exclusively from birth to 3 mo [n=5 per group]
 - Nutritional needs of rhesus infants are similar to those of human infants
- Formula-fed monkeys had:
 - More rapid growth and higher serum insulin
 - Higher levels of bacteria from the *Ruminococcus* genus and lower levels of bacteria from the *Lactobacillus* genus
 - Elevated levels of many cytokines, chemokines, and growth factors at 4 wk of age



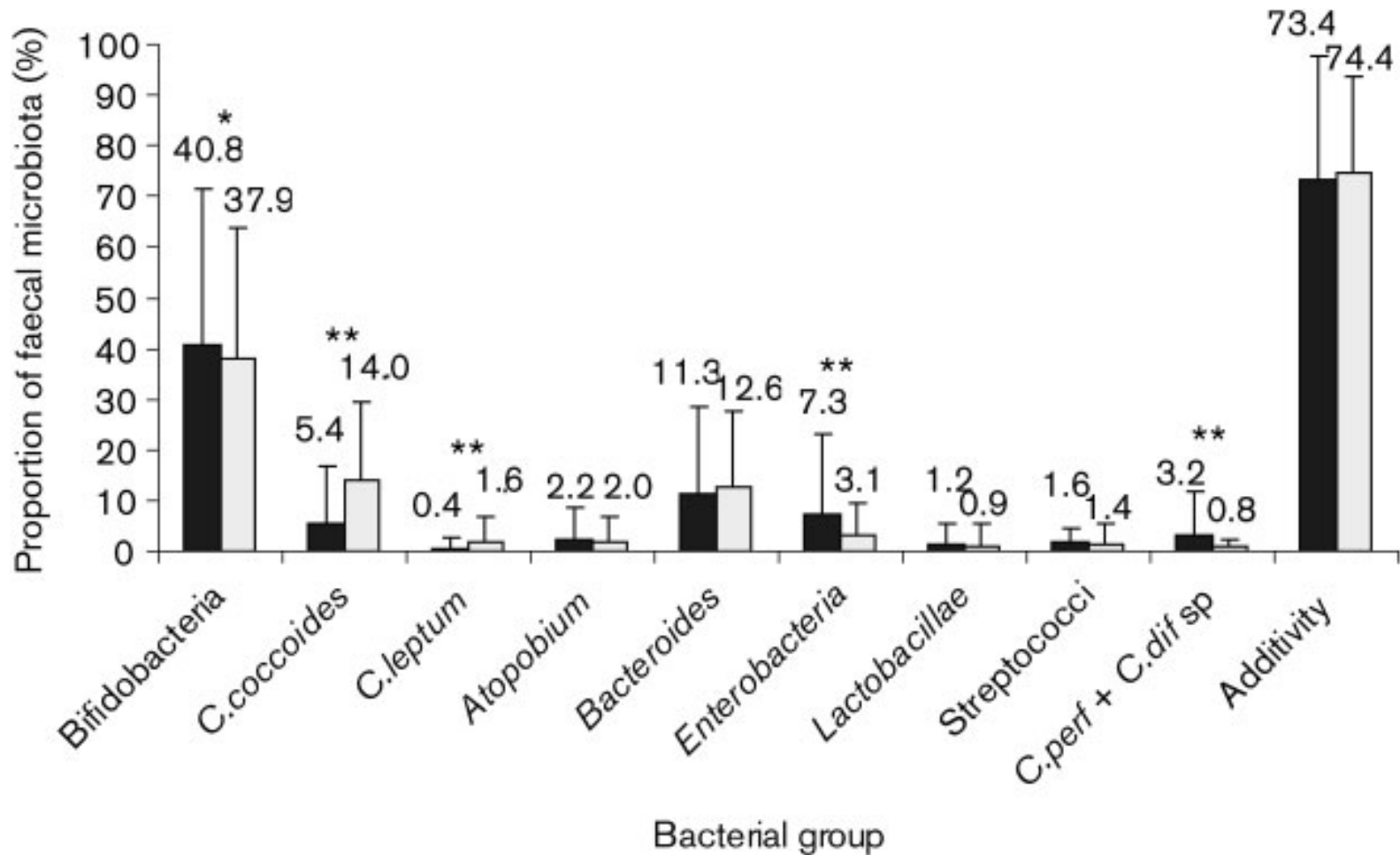
Principal Component Analysis (PCA) reveals distinct separation between serum and urine metabolomic profiles from breast-fed (green), formula-fed (red) infant rhesus monkeys, as well as measurements collected at birth (blue, assigned to formula-fed, yellow assigned to breast-fed) infant rhesus monkeys. (A) Serum NMR data collected from infants from week 2 of life onward for breast-fed ($n = 27$), formula-fed ($n = 30$), as well as serum collected from birth infants ($n = 9$) ($R^2X = 0.67$; $Q^2 = 0.29$). (B) Urine NMR data collected from infants from week 1 of life onward for breast-fed ($n = 54$) and formula-fed ($n = 57$) infants; as well as urine collected from birth infants ($n = 8$) ($R^2X = 0.48$; $Q^2 = 0.22$). In both score plots, the confidence interval is defined by the Hotelling's T^2 ellipse (95% confidence interval).

Introduction of solid foods

Determinants of the human infant intestinal microbiota after the introduction of first complementary foods

[Fallani et al. Microbiology 2011]

- Assessed fecal microbiota composition of infants from 5 European countries (Sweden, Scotland, Germany, Italy and Spain)
- Samples collected from 605 infants at 6 wk of age and ~4 wk after the introduction of solid foods
- At 6 wk, 59% fully BF, 27% fully formula-fed, 14% mixed-fed



Composition of the faecal microbiota of 531 infants before weaning (6 weeks of age) (black bars) and 4 weeks after the introduction of first solid foods (grey bars). Values are mean (\pm SD) proportions of the bacterial groups quantified by FISH-flow cytometry. Asterisks indicate significant differences between the two periods (*P,0.05; **P,0.001).

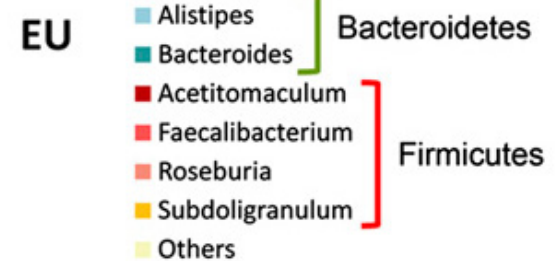
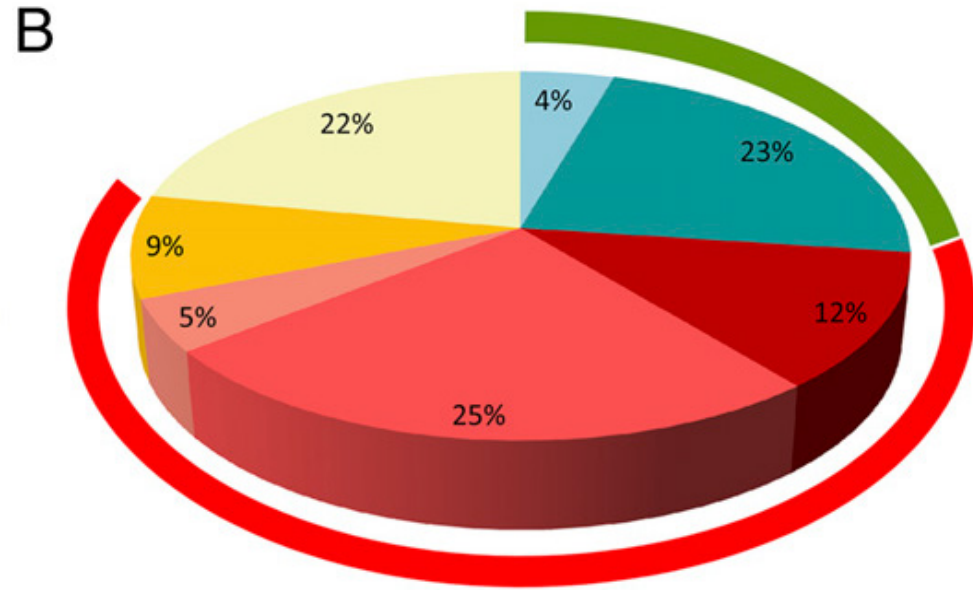
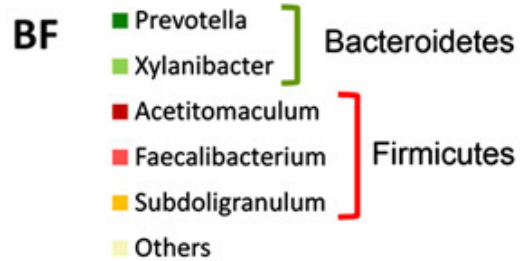
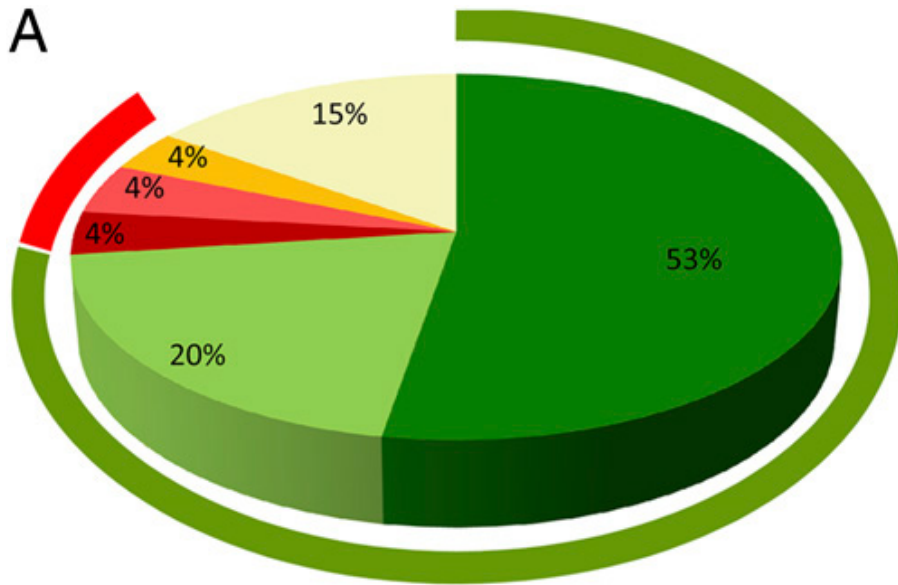
Gut microbiota of children in Burkina Faso vs. Europe diverges after weaning [De Filippo et al. PNAS 2010]

- Burkinabe diet
 - low in fat and animal protein
 - rich in starch, fiber, and plant polysaccharides
 - predominantly vegetarian
- European diet
 - high in fat, animal protein, sugar, starch
 - low in fiber

Gut microbiota of children in Burkina Faso vs. Europe diverges after weaning

[De Filippo et al. PNAS 2010]

- Differences in microbiota became evident after the period of predominant breastfeeding
- Higher microbial richness and biodiversity in Burkina Faso samples than in European samples
- Actinobacteria and Bacteroidetes were more represented in Burkina Faso
- Firmicutes and Proteobacteria were more abundant in European children



Pie charts of median values of bacterial genera present in fecal samples of Burkinabe (BF) and EU children (>3%) found by RDP classifier v. 2.1. Rings represent corresponding phylum (Bacteroidetes in green and Firmicutes in red) for each of the most frequently represented genera.

Gut microbiota of children in Burkina Faso vs. Europe diverges after weaning

[De Filippo et al. PNAS 2010]

- Greater total short chain fatty acids (SCFA) in fecal samples from Burkinabe children (especially propionic and butyric acids)
- SCFA produced when indigestible plant components such as plant polysaccharides are fermented by intestinal microbiota
- Are precursors for gluconeogenesis, liponeogenesis, and protein and cholesterol synthesis
- SCFA-producing bacteria may prevent establishment of potentially pathogenic intestinal microbes
- SCFA have protective role against gut inflammation

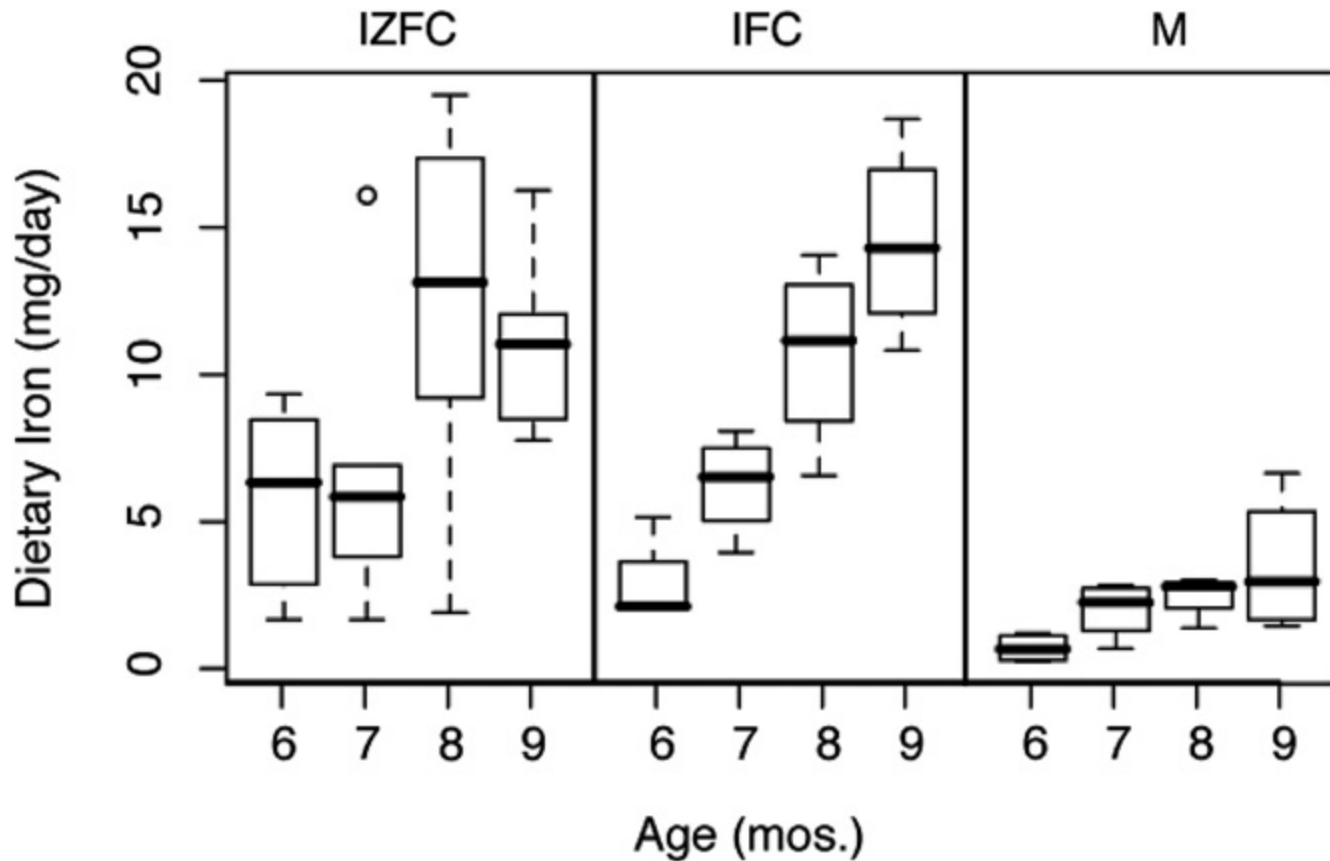
Which nutrients influence infant & child microbiota?

- Iron?
 - Iron is an essential nutrient for many gut microbes, but some beneficial barrier bacteria (e.g. Lactobacilli) do not require iron
 - For most enteric gram-negative bacteria (e.g., Salmonella, Shigella, or pathogenic Escherichia coli), iron acquisition plays an essential role in the virulence and colonization of most pathogenic strains [Zimmermann et al., 2010]
- Fatty acids?
 - n-3 LCPUFA may modulate growth and adhesion of lactobacilli; may impair growth of Bacteroides thetaiotaomicron

Effects of different complementary feeding regimens on iron status and enteric microbiota in breastfed infants

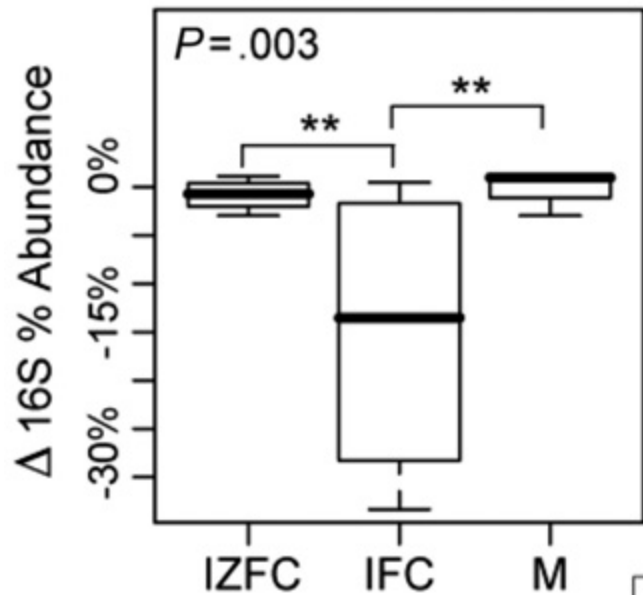
[Krebs et al. J Pediatr 2013]

- 45 exclusively breastfed 5-month-old infants were randomized to 1 of 3 feeding groups
 - Commercially available pureed meats
 - Iron- and zinc-fortified infant cereals
 - Iron-only fortified infant cereals
- Followed to 9-10 mo of age
- Enteric microbiome analyzed for last 14 infants enrolled
- Fecal samples obtained monthly, 5-9 mo
- Cereal groups had higher iron intake

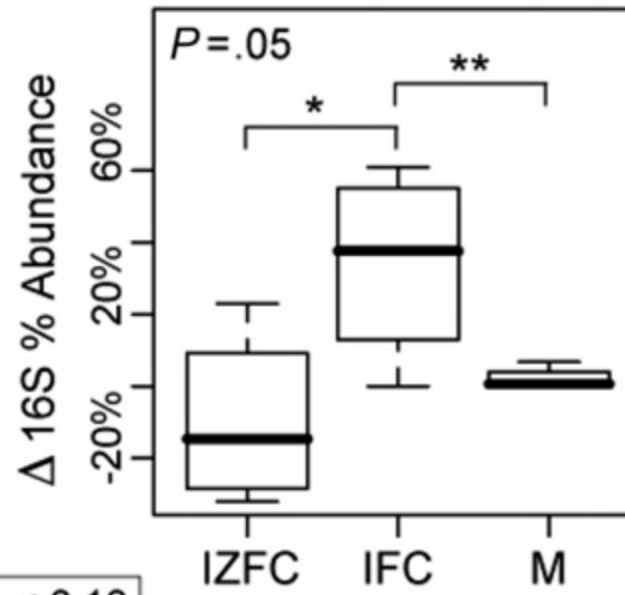


Longitudinal iron intake (mg/day) by feeding group. Total dietary iron was significantly higher in the 2 cereal FGs compared with the meat FG at each time point ($P = .01, .002, .0003, \text{ and } .0001$ at 6, 7, 8, and 9 months, respectively).

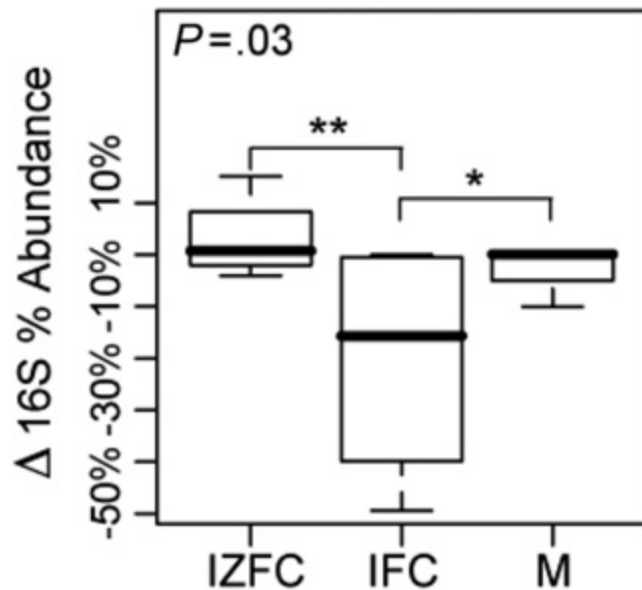
Actinobacteria



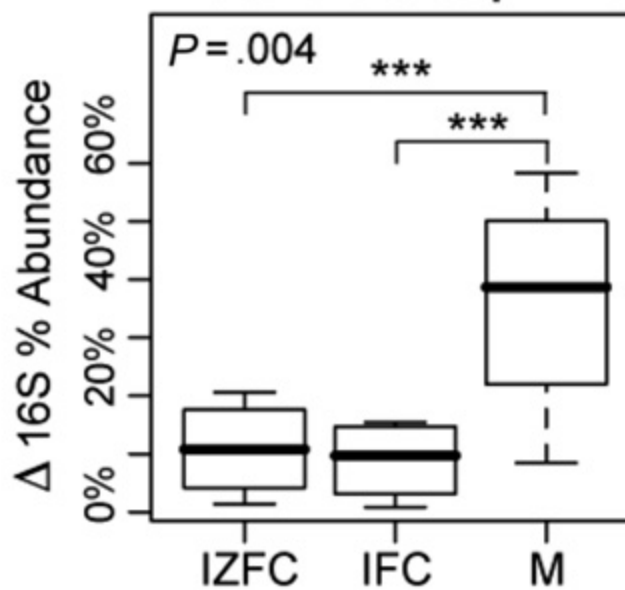
Bacteroidetes



Firmicutes Bacilli



Firmicutes Clostridia Group XIVa



* < 0.10
** < 0.05
*** < 0.01

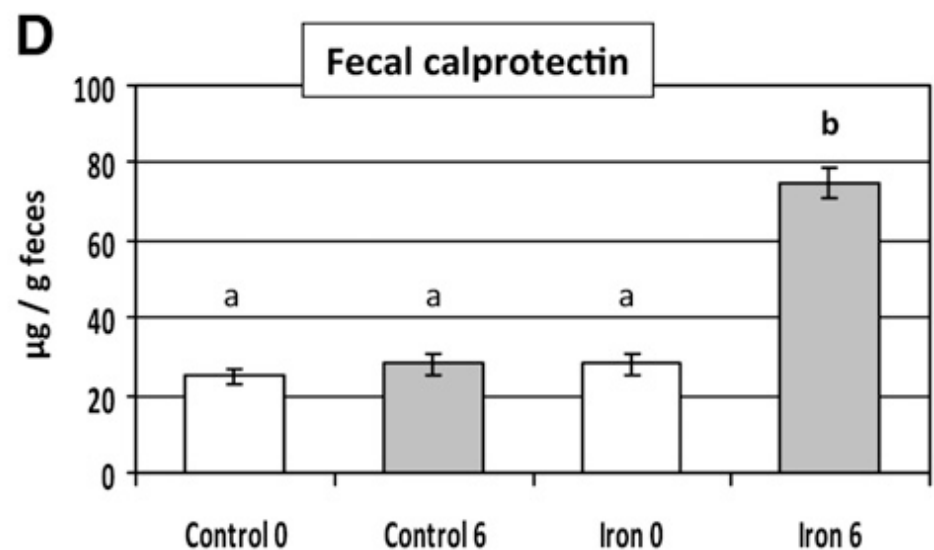
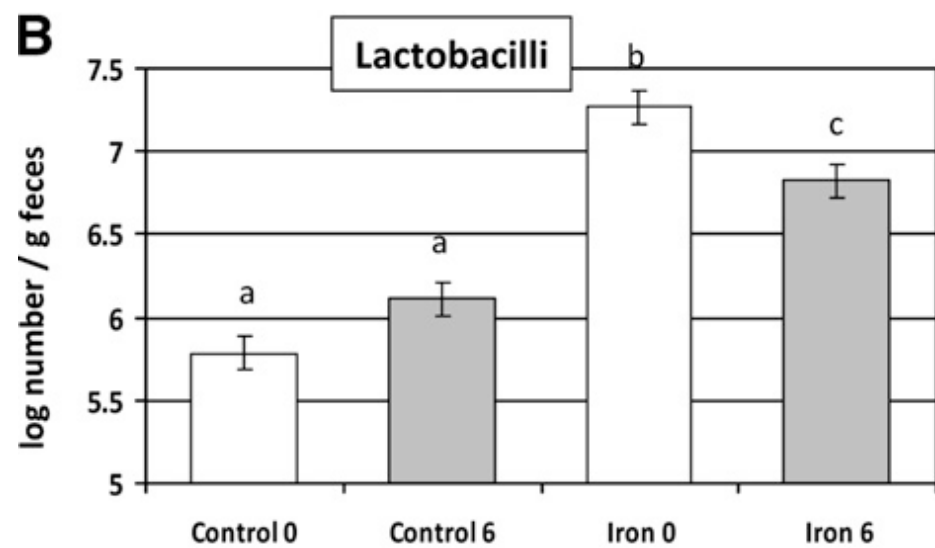
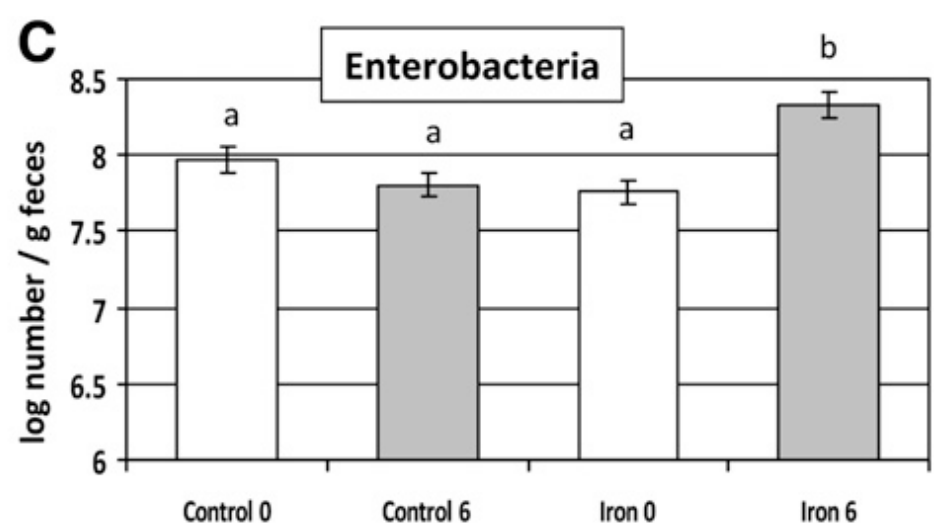
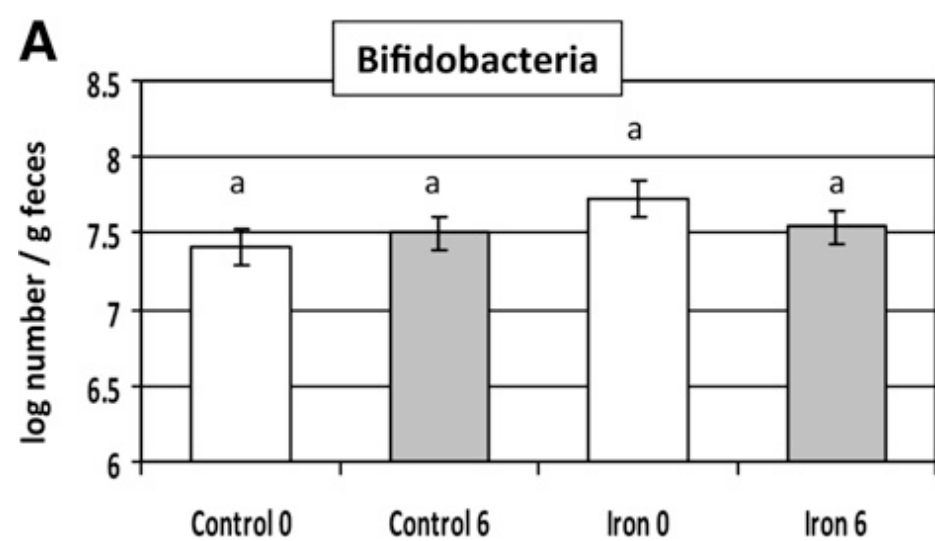
- Actinobacteria (incl. Bifidobacteria and Rothia) & Lactobacillales decreased over time in infants receiving iron-fortified cereal; Bacteroides more abundant in that group

- Clostridia group XIVa clade increased in meat group

The effects of iron fortification on the gut microbiota in African children

[Zimmermann et al. Am J Clin Nutr 2010]

- Double-blind RCT in Cote d'Ivoire
- 6– 14-y-old children (n = 139) received either iron-fortified or non-fortified biscuits for 6 mo
- Iron-fortified biscuits contained 20 mg Fe/d, 4 times/wk as electrolytic iron

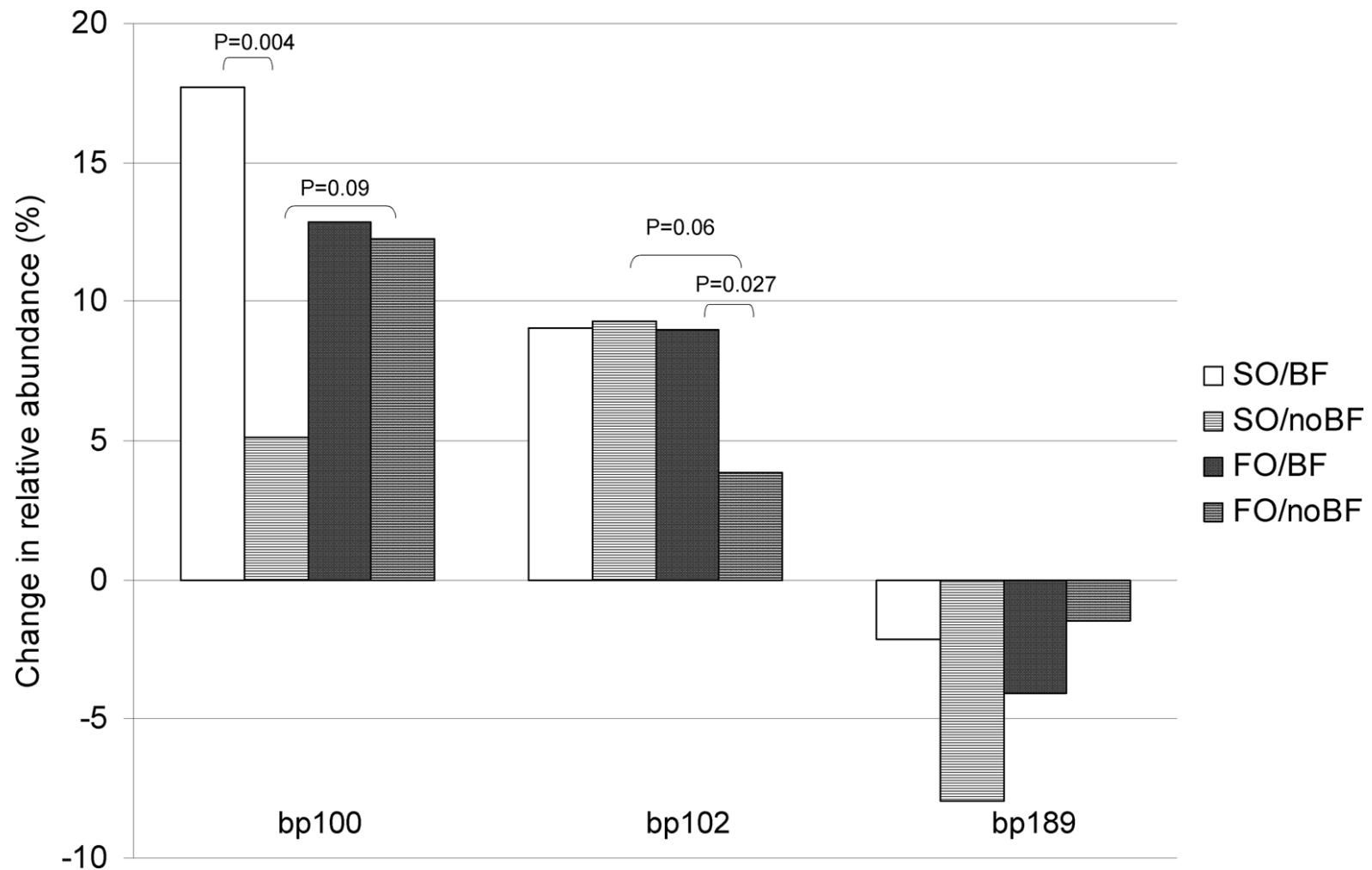


Iron fortification produced a potentially more pathogenic gut microbiota profile, and this profile was associated with increased gut inflammation [Zimmermann et al., 2010]

Effect of fish oil supplements on fecal microbiota from 9 to 18 mo

[Andersen et al. JPGN 2011]

- Supplementation with 5mL of fish oil (FO) or sunflower oil (SO) from 9 to 18 months of age
- Stool samples were collected from 132 healthy Danish infants
- Molecular fingerprints of the bacterial DNA were obtained by terminal restriction fragment length polymorphism (T-RFLP).
- Effects on bp100 and 102 (both presumed to be Bacteroidetes) were modified by BF status



Among infants **not breastfed** at 9 mo:

Greater increase in bp100 in the fish oil (FO) group

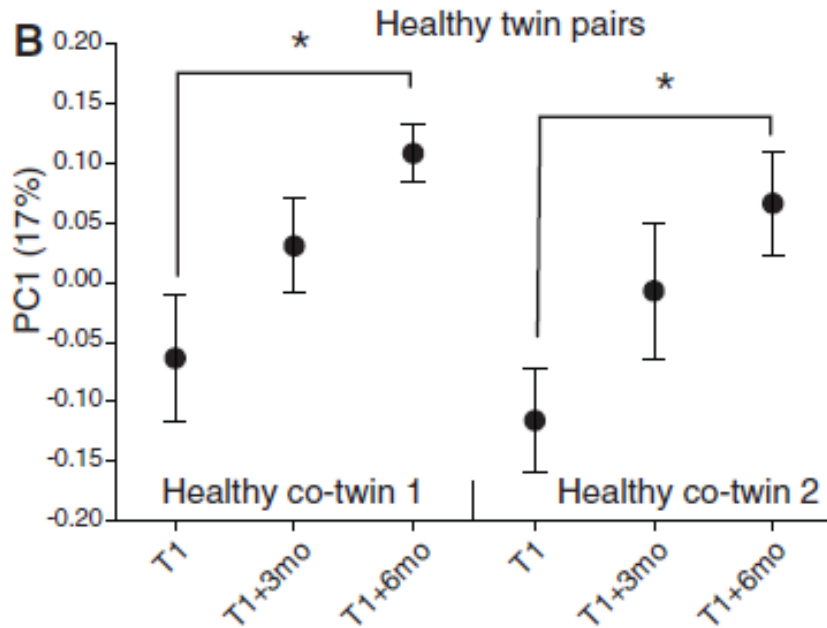
Greater increase in bp102 in the sunflower oil (SO) group

How does the microbiome influence
child nutritional status?

Gut microbiomes of Malawian twin pairs discordant for kwashiorkor

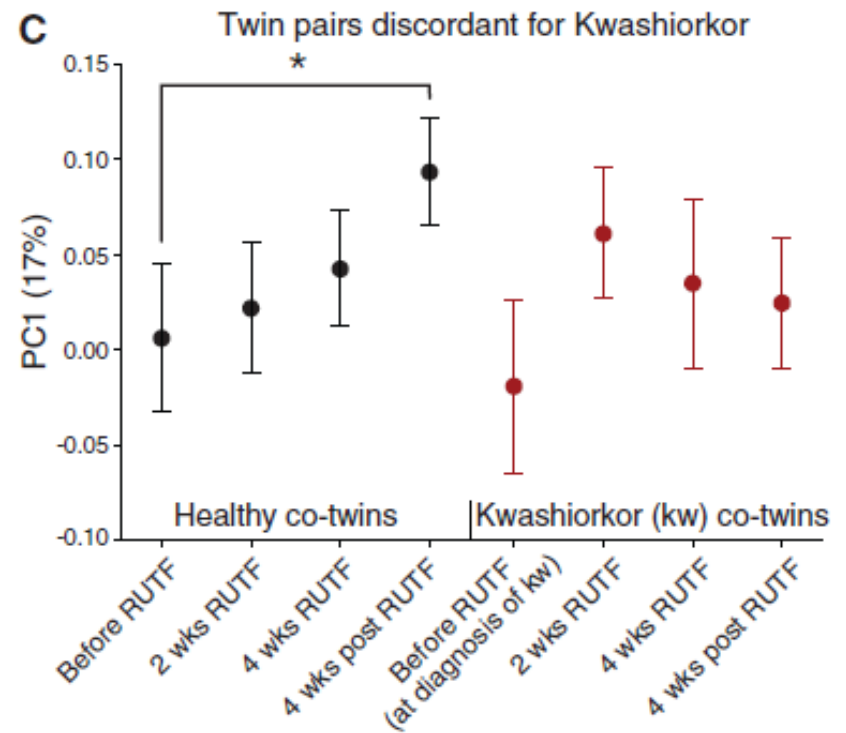
[Smith et al. Science 2013]

- 317 twin pairs followed during the first 3 y of life
 - 50% of the twin pairs did not develop acute malnutrition (kwashiorkor, marasmus or moderate acute malnutrition)
 - 43% became discordant
 - 7% manifested concordance for acute malnutrition
- Both children in twin pairs discordant for kwashiorkor were treated with a peanut-based, ready-to-use therapeutic food (RUTF)
- Assessed microbiomes of 9 same-gender twin pairs without malnutrition & 13 pairs discordant for kwashiorkor



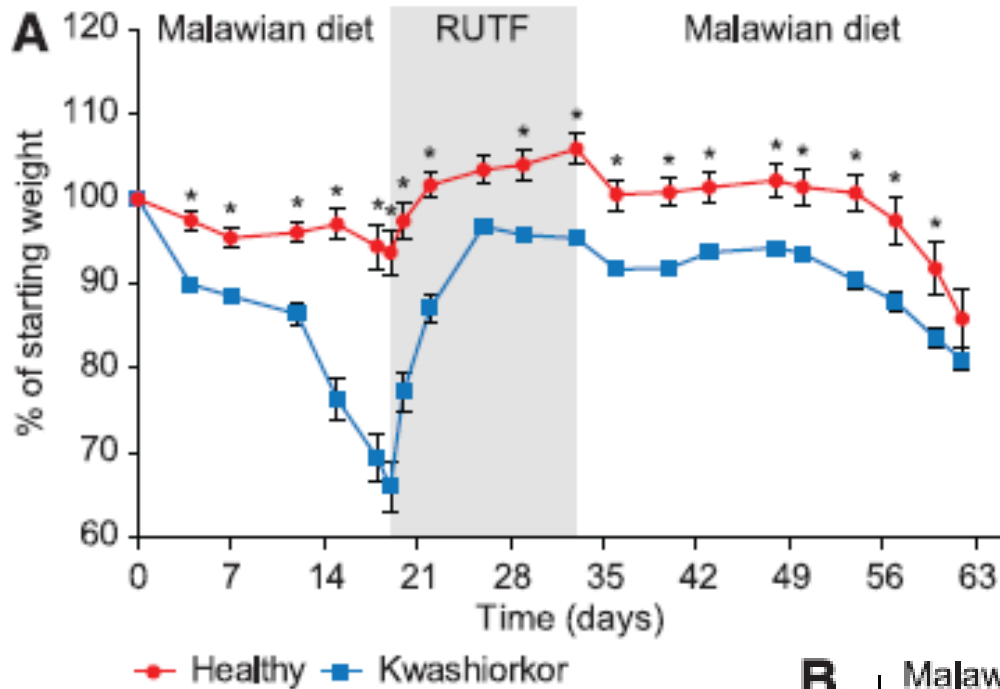
Microbiomes of twins with kwashiorkor are less 'mature' compared to their non-malnourished co-twins

Smith et al., 2013



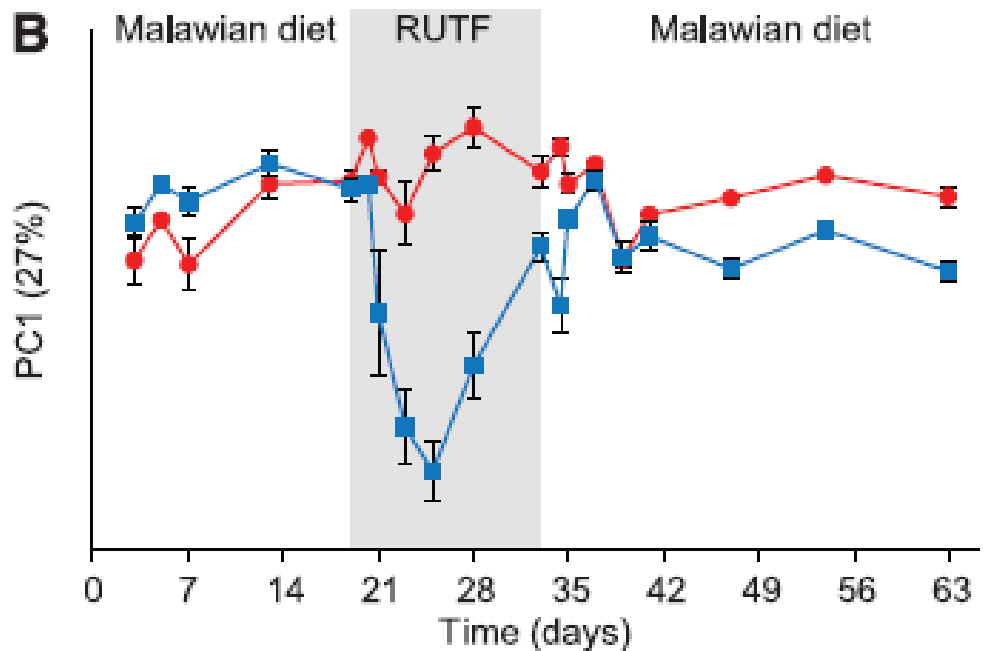
Humanized gnotobiotic mice (“personalized” gnotobiotics)

- Previously frozen fecal communities from several discordant pairs were each transplanted into gnotobiotic mice
 - Replicate a person’s gut microbiota in multiple recipient animals who are given diets resembling those of the human microbiota donor
 - Mice followed over time under highly controlled conditions
 - Gnotobiotic mouse recipients can transmit their human gut microbiota to their offspring (intergenerational transfer)



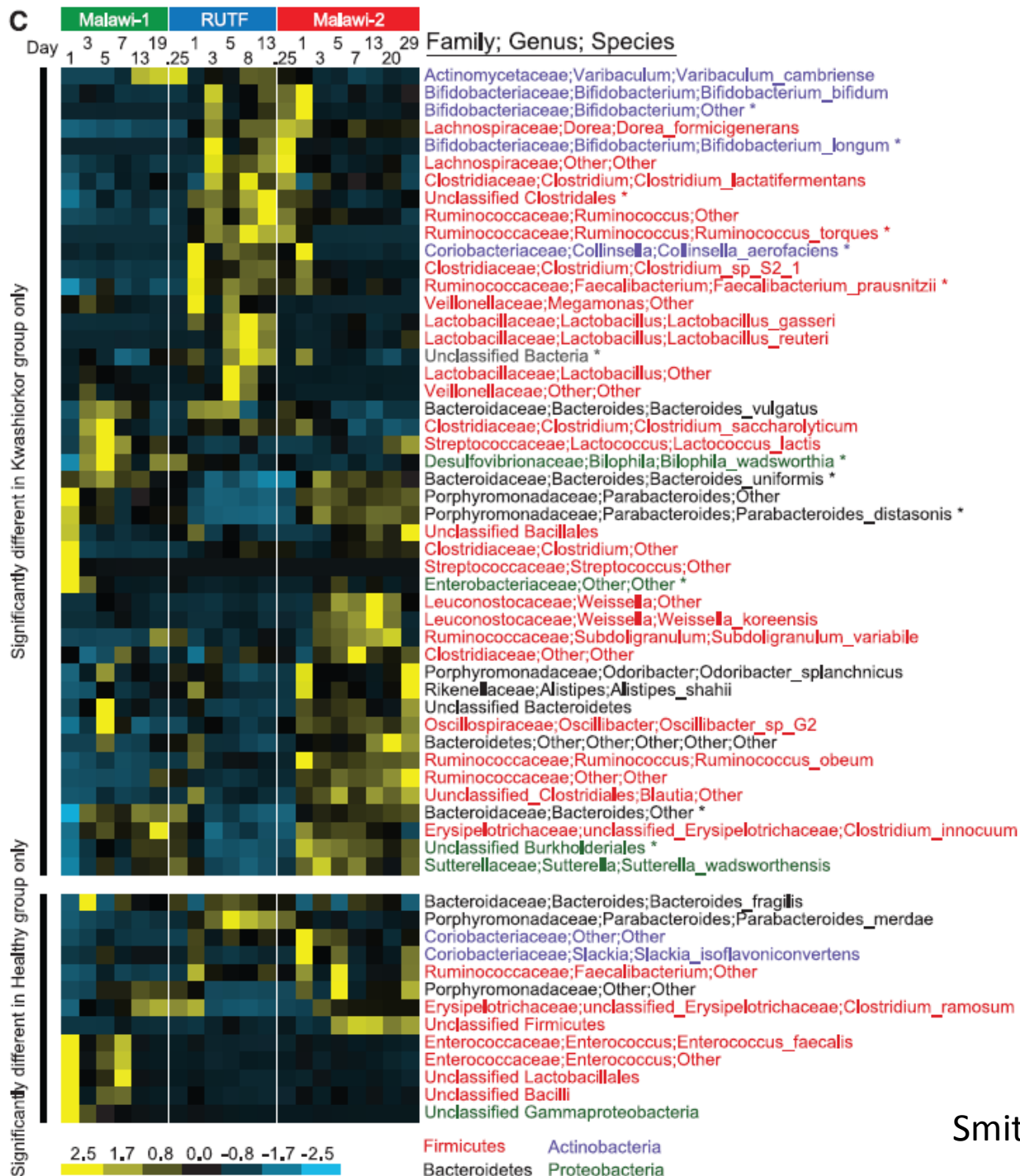
A: Weight loss in mice that received fecal microbiota from co-twin with kwashiorkor vs. non-malnourished co-twin (n=10 mice per group)

B: Fecal microbiota (PC1 coordinate) in mice that received fecal microbiota from co-twin with kwashiorkor vs. non-malnourished co-twin (n=10 mice per group)



Switch from Malawi diet to RUTF

- 30 species level taxa exhibited significant changes in their representation in **kwashiorkor microbiota** transplant recipients
 - Increases in *Bifidobacteria* (*B. longum*, *B. bifidum*), two *Lactobacilli* (*L. reuteri* and *L. gasseri*), *R. torques* and *Faecalibacterium prausnitzii*
 - Decreases in Bacteroidales (*B. uniformis*), *Parabacteroides distasonis*, plus an unclassified *Parabacteroides* taxon
- 28 species level taxa exhibited significant changes in their representation in **healthy co-twins** microbiota transplant recipients



Where do we go from here?



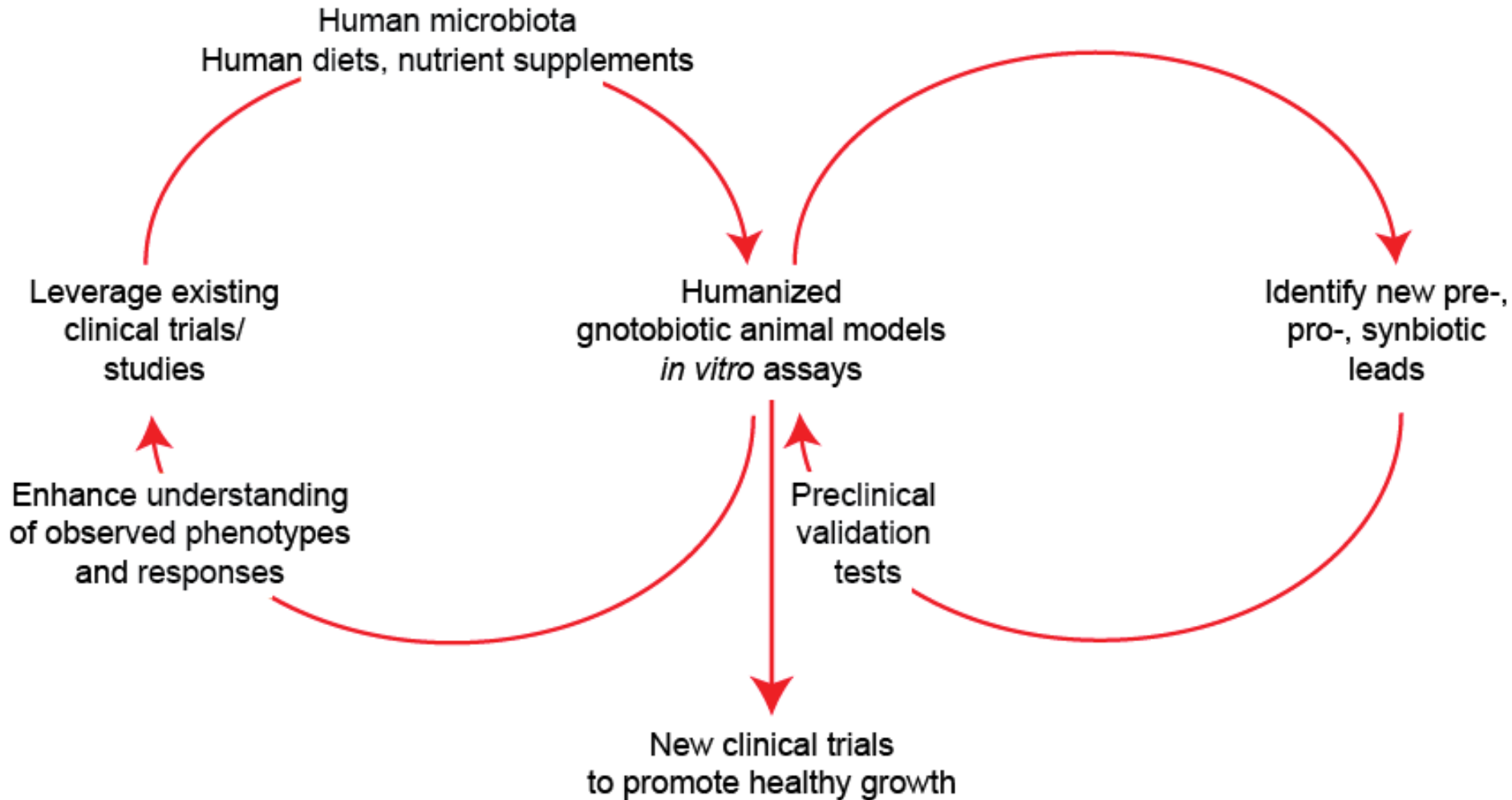
The **B**reast **M**ilk, gut **M**icrobiome, and **I**mmunity (**BMMI**) Project: discovering new ways to promote healthy growth in infants and children



Goals of the BMMI Project

- Identify and validate new pre- and probiotic interventions to improve the health and development of infants and children in the developing world
- Demonstrate an effective process by which new such interventions can be identified and validated in the future

'Central Metabolism' of the BMMI Project



Gaps identified

- Apart from studies comparing breastfed and formula-fed infants, there is very little information on how dietary composition or nutrient intake affects the microbiome of children.
- The etiology of the emerging link between malnutrition and the microbiome, and the prevention or treatment of abnormal status via dietary or pre/probiotic interventions, require further investigation.
- Prospective studies, particularly long-term follow-up of intervention trials, are needed to identify the short- and long-term consequences of differences in microbiome structure and function in early life.



BMMI Team at the Bill & Melinda Gates Foundation, Feb 2012

Thank you