CHILD Study
HELP CHILDREN GROW UP HEALTHY

DEVOTION
Developmental Origins of Chronic Diseases in Children Network

University of Manitoba

the Children's Hospital Research Institute of Manitoba

Role of Early Life Environment in Shaping the Gut Microbiota

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OCTOBER 4, 2010
Environment Special:
The oceans—why 70%
of our planet is in danger
The Facebook Movie:
The secret history of
social networking
TIME
How the first nine months shape the rest of your life
The new science of fetal origins
BY ANNIE MURPHY PAUL
www.time.com


The first
100
days

“Developmental Origins”

- **1990s: Fetal Origins of Adult Disease (FOAD)**

  Environmental exposures during fetal life influence adult health

- **2000s: Developmental Origins of Health and Disease (DOHaD)**

  Both the prenatal and postnatal environment shape developmental trajectories that influence health throughout the lifecourse

Developmental Origins of…

Allergies

1 in 4 Canadians have seasonal allergies

1 in 13 have food allergies

Canadian Allergy, Asthma and Immunology Foundation & 2013 SCAAALAR survey

Asthma

1 in 6 Canadian children have asthma

Public Health Agency of Canada (2007). Life and breath: Respiratory disease in Canada.

Obesity

1 in 3 Canadian children are overweight

Overweight and obesity in children and adolescents: Results from the 2009 to 2011 Canadian Health Measures Survey

DOHaD: What are the important early-life exposures?

The Canadian Healthy Infant Longitudinal Development (CHILD) Study

How do genes and the environment influence child health and development?

CIHR IRSC
Canadian Institutes of Health Research | Instituts de recherche en santé du Canada

AllerGen
Allergy, Genes and Environment Network
Le réseau des allergies, des gènes et de l'environnement

$30M	Invested
500,000	Samples: Blood, Urine, **Stool**, Nasal Swabs, Dust, **Breast Milk**
200,000	Questionnaires
3600	Families
40+	Researchers
20+	Disciplines
5(+)	Years Follow-Up
93%	Retention

CHILD Study

HELP CHILDREN
GROW UP HEALTHY

PRENATAL EXPOSURES

POSTNATAL EXPOSURES

- Home Environment (Chemicals, Tobacco Smoke, Pets, etc.)
- Air Pollution (GIS Modelling)
- Maternal Nutrition
- Child Nutrition
- Breastfeeding
- Viral Infections
- Maternal Stress

Prenatal 2009-2012	Birth	3 month	12 month	3 year	5 year
	Clinic Visit	Hospital Data	Home Visit	Clinic Visit	Clinic Visit	Clinic Visit
			 			

Perinatal Exposures

- **Cesarean Section** (WHO 2013)
  - Brazil 56%, USA 33%, Canada 27%, Sweden 17%

- **Intrapartum Antibiotics** (CDC)
  - 25% of US population (1 million women annually) exposed for GBS prophylaxis

- **Infant Feeding:** (CDC 2008)
  - WHO recommends: exclusive breastfeeding for 6 months, continued BF to 2 years+
  - Most US infants initiate breastfeeding, BUT
    - Within the first week, >50% are receiving formula
    - By 6 months, <50% are breastfed at all

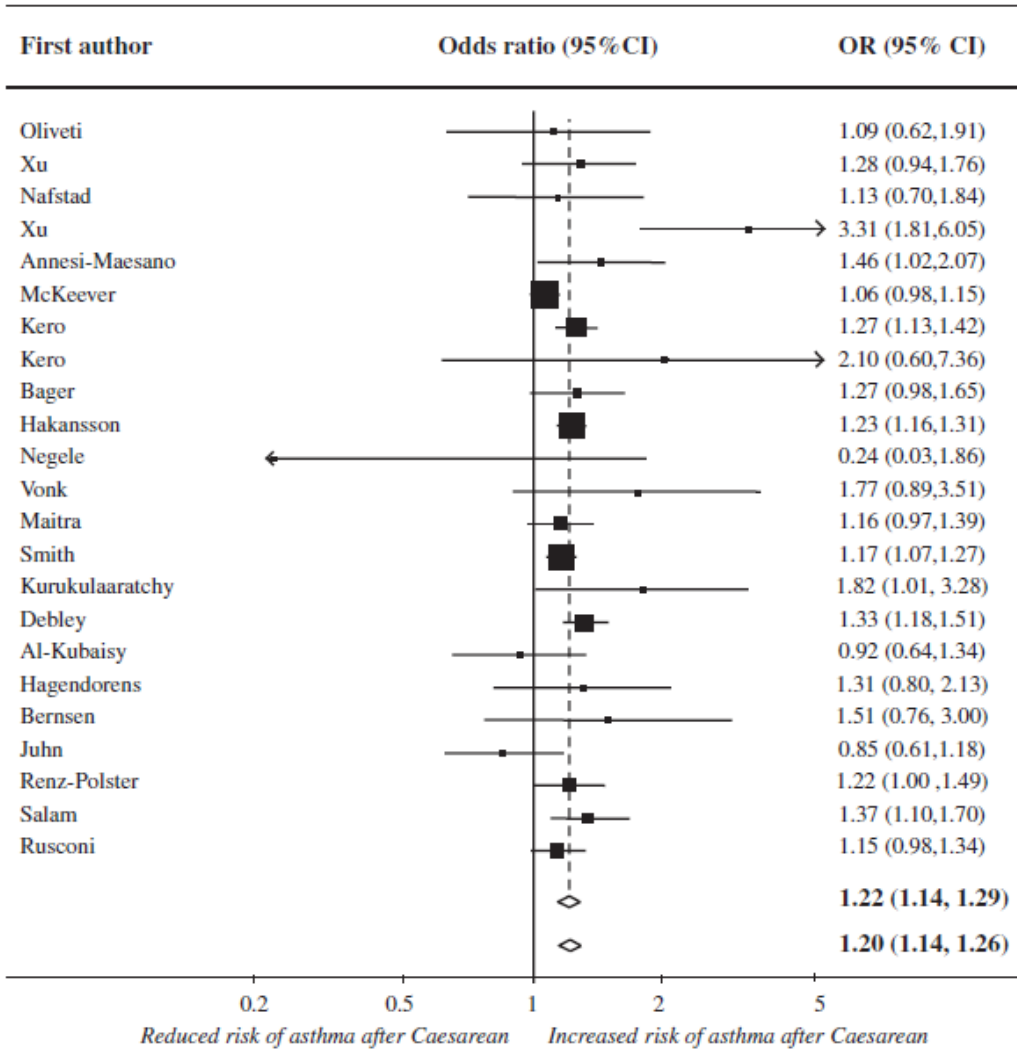
Asthma?

Cesarean Section & Asthma

Meta-analysis of
23 studies:

20% increased risk
in children delivered by
Cesarean section.

(Thavagnanam et al. *Clin Exp Allergy*. 2008 38:4)



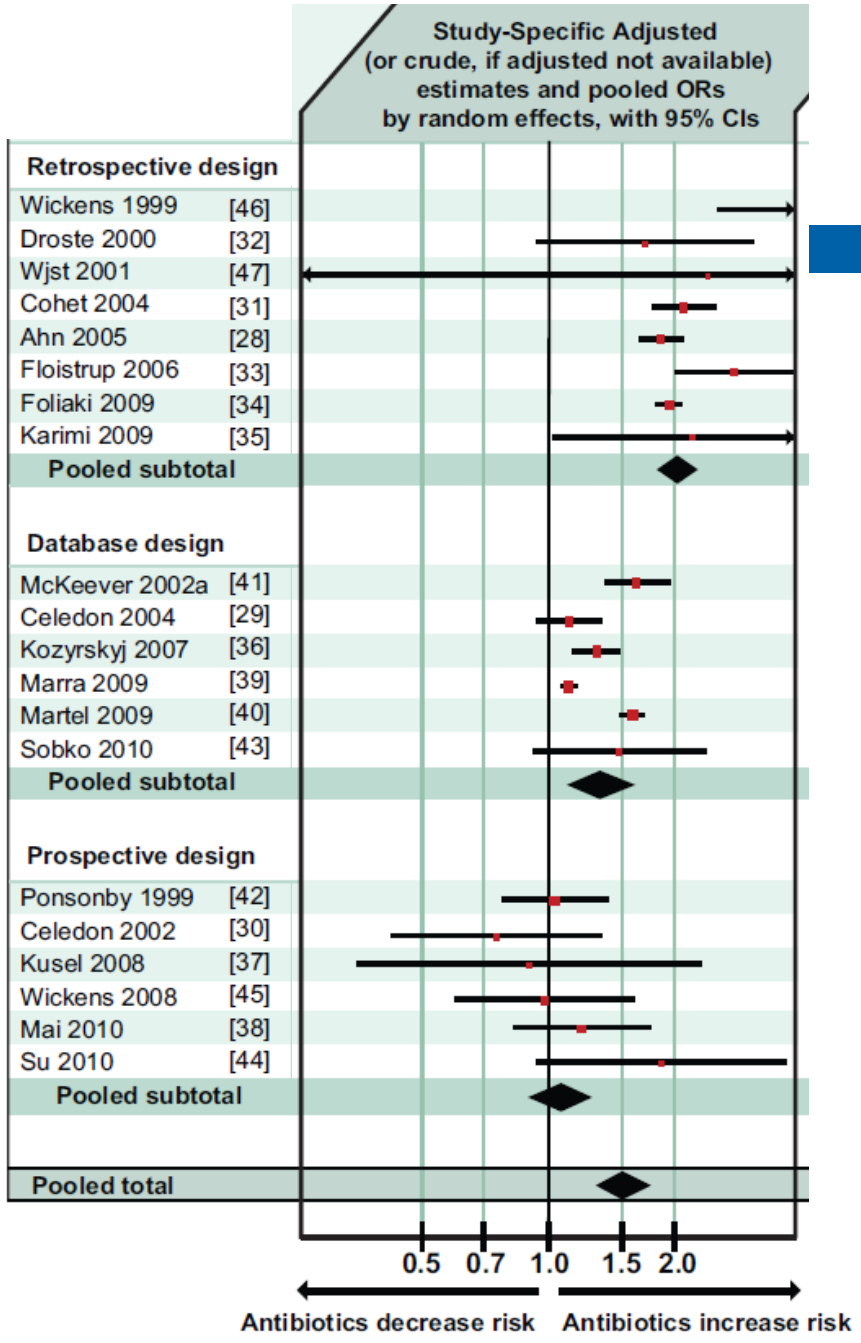
Antibiotics & Asthma

Meta-analysis of
20 studies:

50% increased risk
following infant antibiotic* exposure

(Murk et al. 2011)

*Few studies on intrapartum antibiotics

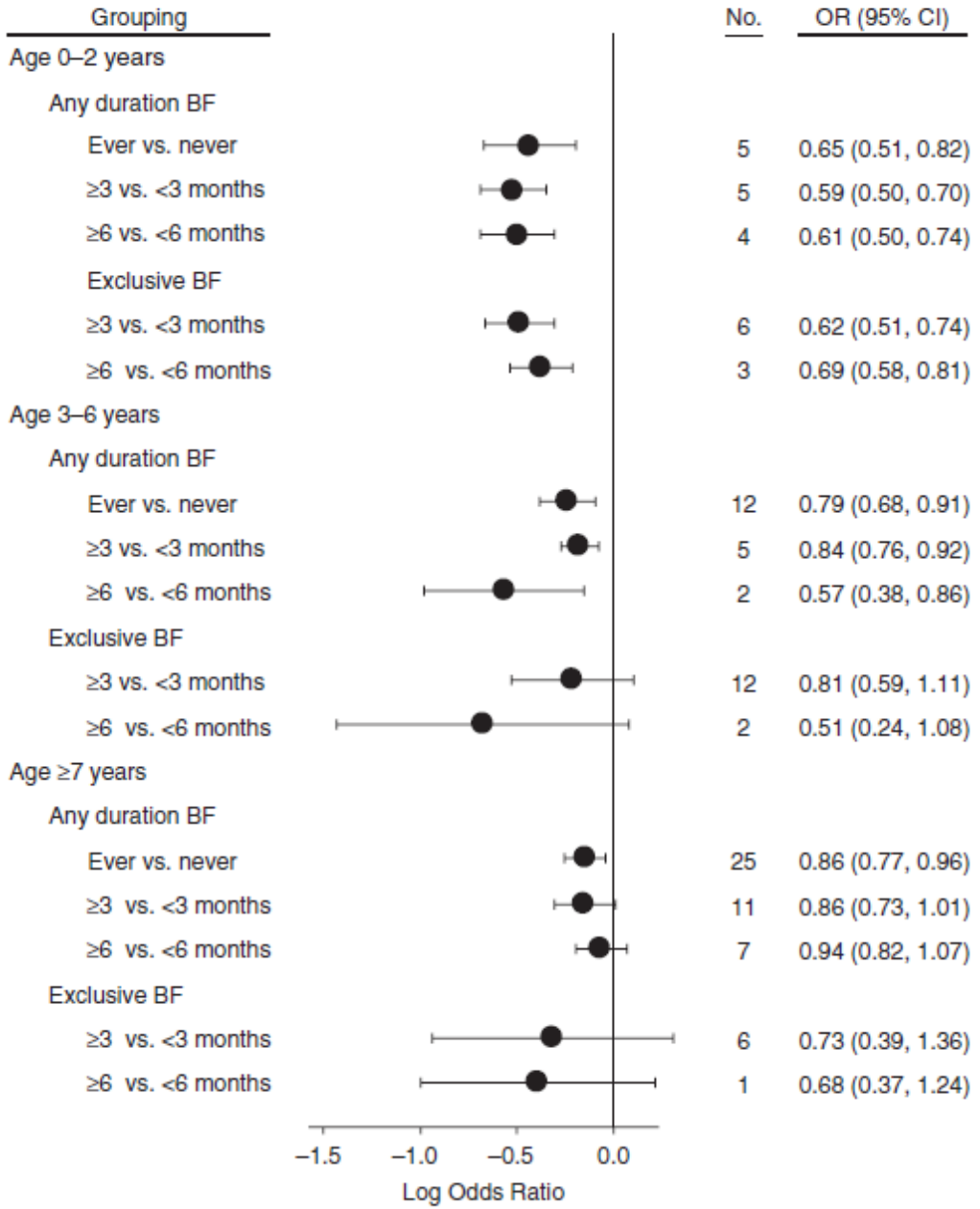


Breastfeeding & Asthma

Meta-analysis of
117 Studies:

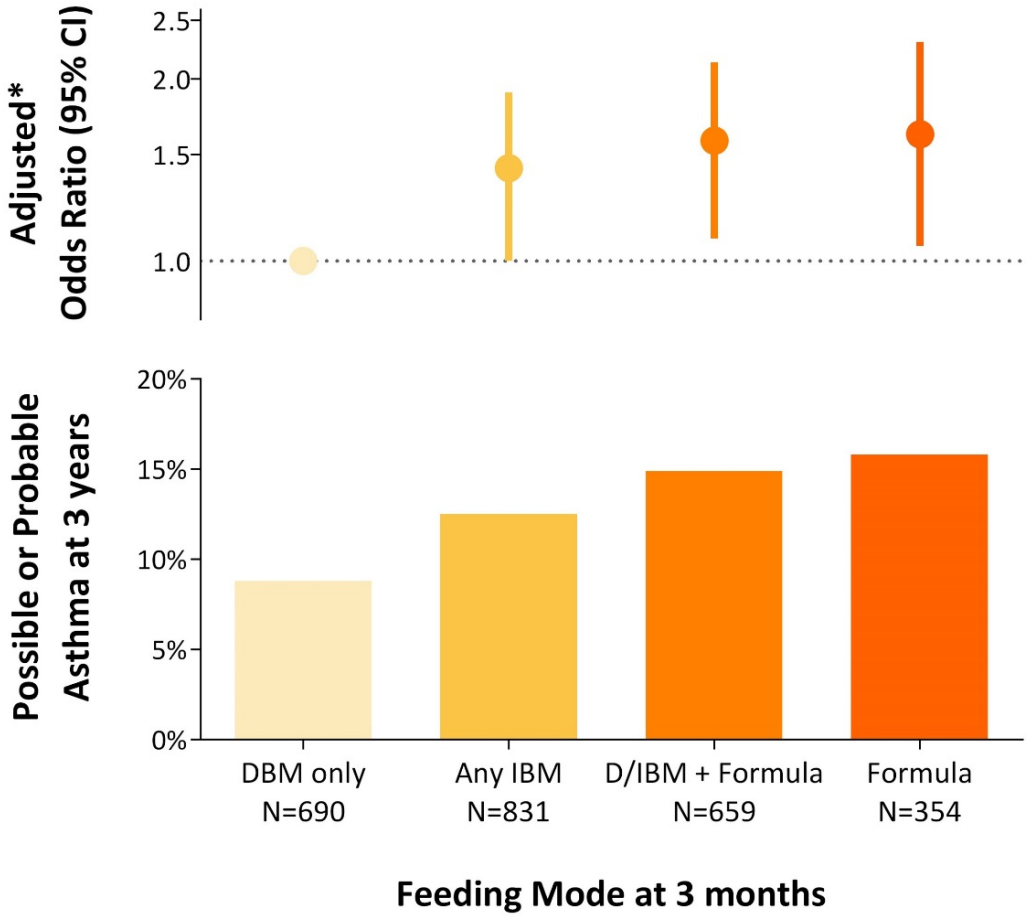
~30% reduced risk
in breastfed infants

(Dogaru et al. *AJE* 2013)



("Asthma ever")

Breast(milk)feeding & Asthma



*Adjusted for infant sex, maternal diagnosis of asthma, ethnicity, method of birth, daycare attendance, gestational age and solid food introduction; with multiple imputation of missing data.

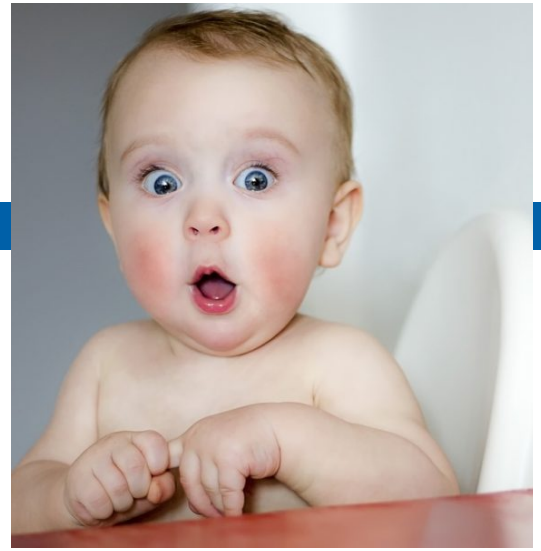
DBM = Direct Breast Milk
IBM = Indirect (pumped) Breast Milk

Compared to direct breastfeeding, **any other mode of infant feeding** was associated with an **increased risk** of possible or probable asthma by 3 years of age.

- Bioactivity of milk?
- Milk/skin microbiota?
- Physical lung exercise?
- Infant→Mother signalling?
- Toxins from bottles?

(Klopp et al. *J Pediatrics* 2017)

Early Life Exposures



- **Pets** during infancy
  - 66% ↓ risk of asthma (age 12) (Hesselmar et al. *Clin Exp Allergy* 1999)
- **Tobacco smoke** exposure; prenatal and postnatal:
  - 22% ↑ risk of asthma (age 6+) (Silvestri et al. *Pediatri Pulmonol* 2015)
- **Maternal depression / anxiety:**
  - 25% ↑ risk of asthma (age 7) (Kozyrskyj et al. *Am J Respir Crit Care Med.* 2008 177:2)

DOHaD: Asthma, Allergies & Obesity

- Early **risk** factors:
  - Cesarean section
  - Antibiotics
  - Tobacco smoke
  - Maternal stress
- Early **protective** factors:
  - (Direct) Breastfeeding
  - Pets

?

Biological Mechanisms?

Gut Microbiota

- **Complex "super organ" of ~ 100 trillion commensal microbes living in the gastrointestinal tract**

- Prevent colonization by pathogens
- Educate the developing immune system
- Influence nervous system: 'gut-brain-axis'
- Contribute to host metabolism
  - Digestion of complex carbohydrates
  - Vitamin production
  - Energy harvest

100 TRILLION

The human microbiome is made up of more than 100 trillion bacteria, fungi, protozoa, and viruses that live on and inside the body.

10X

We have 10 times more microbial cells in our body than human cells and the majority live in our guts—especially the large intestine, or colon.

The bacteria in our microbiomes are essential to human health and aid in biological processes such as:

- $E=mc^2$ — Extracting energy from food
- RETINOL, FOLATE, RIBOFLAVIN, BIOTIN, NIACIN — Producing essential vitamins
- Regulating our immune system
- Regulating our glucose levels and metabolism
- Protecting us against disease-causing microbes

SYMBIOTIC

The beneficial and symbiotic relationship between humans and our microbiomes has likely evolved and changed throughout human development.

Personal microbial communities shift throughout a person's life and are influenced by diet, exercise, medications such as antibiotics, pathogens, and other environmental factors.

http://www.serestherapeutics.com/our-science/microbiome-101

Microbiota & **Asthma**

RESEARCH ARTICLE www.ScienceTranslationalMedicine.org 30 September 2015 Vol 7 Issue 307 307ra152

ASTHMA

Early infancy microbial and metabolic alterations affect risk of childhood asthma

Marie-Claire Arrieta,[1,2]* Leah T. Stiemsma,[2,3]* Pedro A. Dimitriu,[2] Lisa Thorson,[1] Shannon Russell,[1,2] Sophie Yurist-Doutsch,[1,2] Boris Kuzeljevic,[3] Matthew J. Gold,[4] Heidi M. Britton,[1] Diana L. Lefebvre,[5] Padmaja Subbarao,[6,7] Piush Mandhane,[8,9] Allan Becker,[10] Kelly M. McNagny,[4] Malcolm R. Sears,[5] Tobias Kollmann,[3,11] the CHILD Study Investigators,[†] William W. Mohn,[2] Stuart E. Turvey,[3,11‡§] B. Brett Finlay[1,2,12‡§]

Microbiota & **Asthma**

RESEARCH ARTICLE www.ScienceTranslationalMedicine.org 30 September 2015 Vol 7 Issue 307 307ra152

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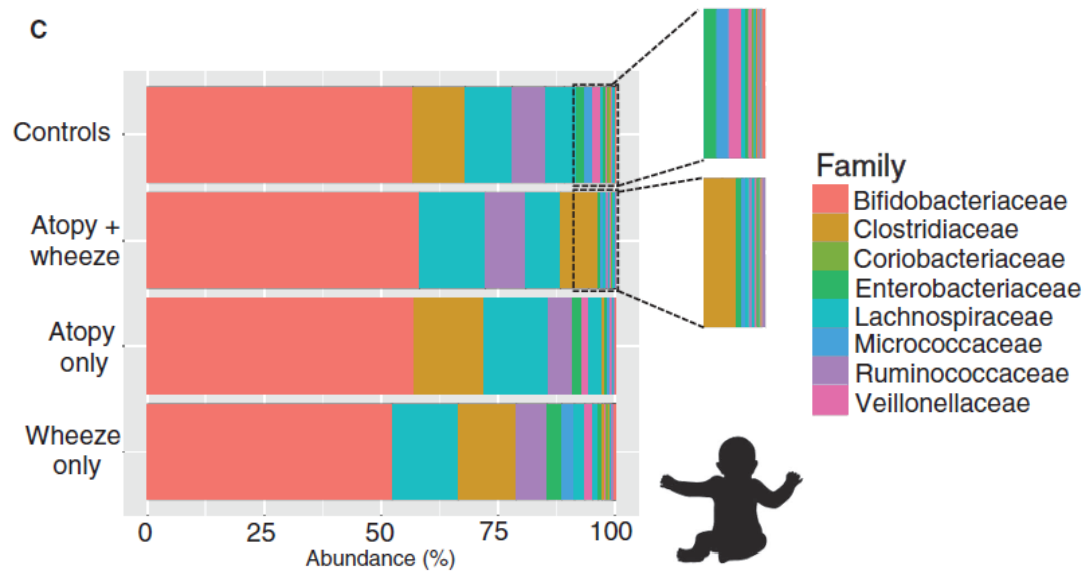
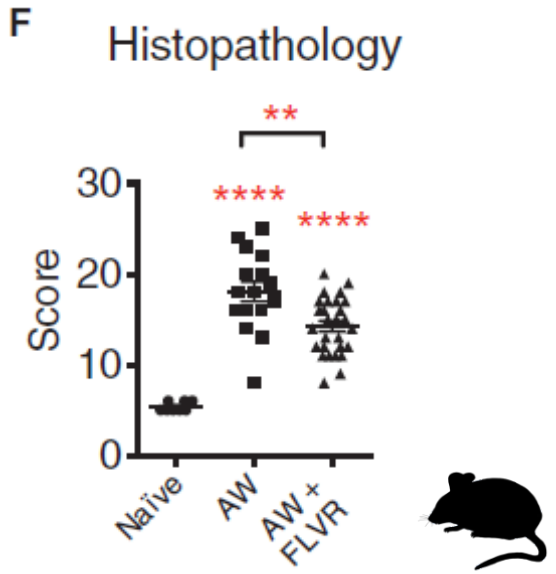
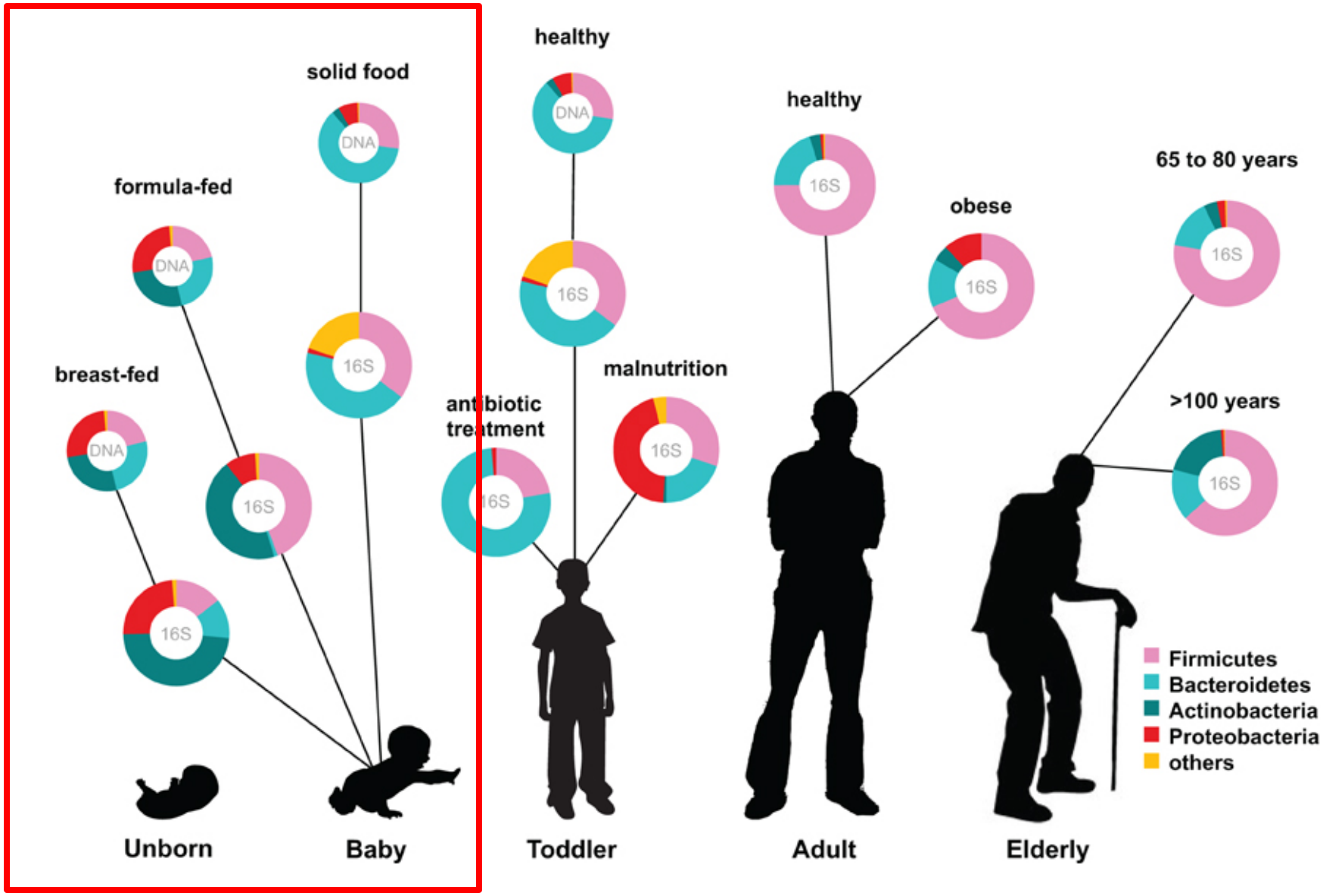
"Infants at risk of asthma exhibited transient gut microbial dysbiosis during the **first 100 days of life."**

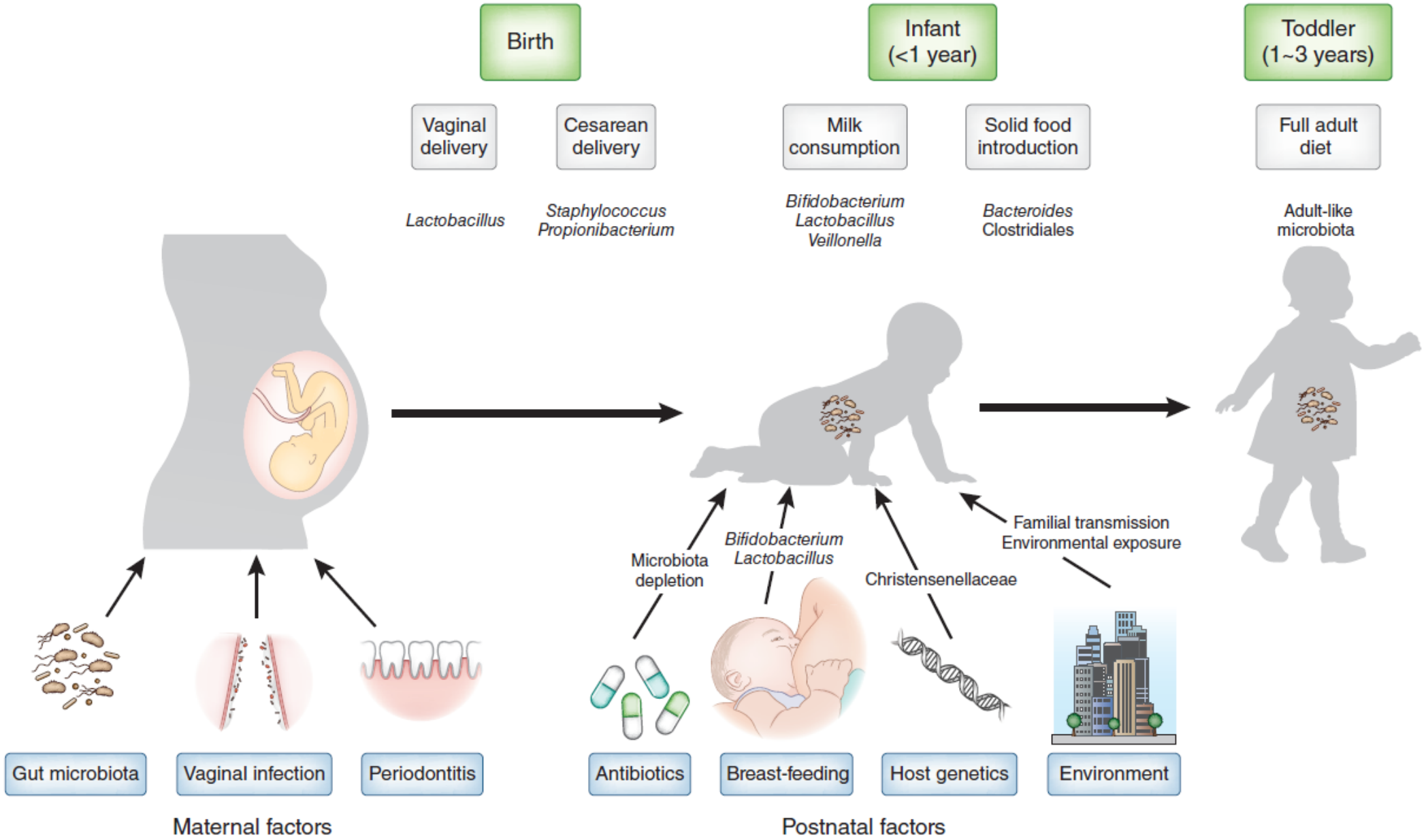






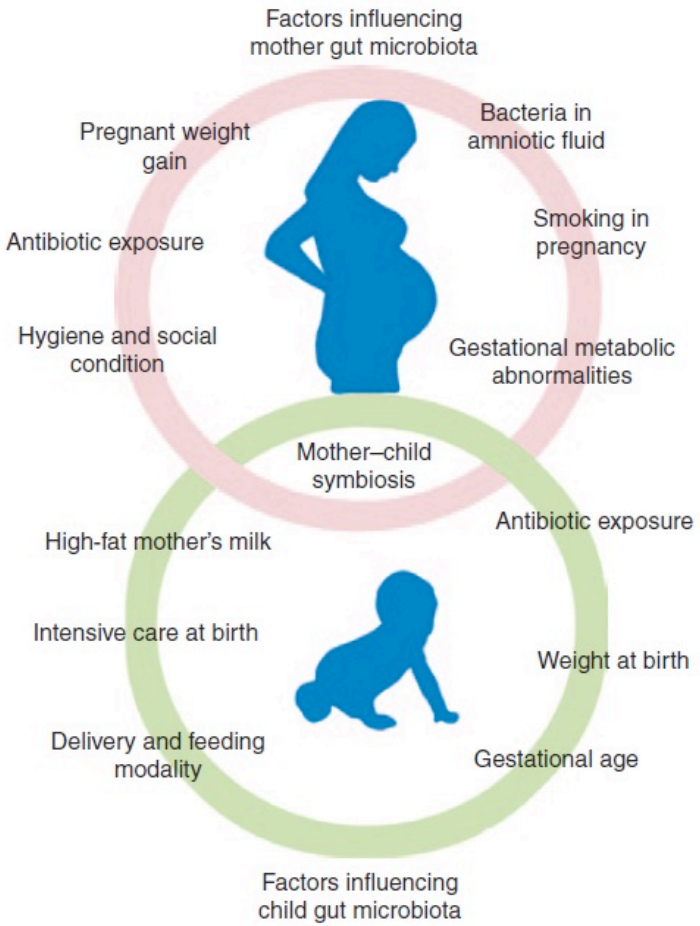
Human microbiota: onset and shaping through life stages and perturbations.
(Ottman et al. *Front Cell Infect Microbiol* 2012)

What early life exposures shape the gut microbiome?

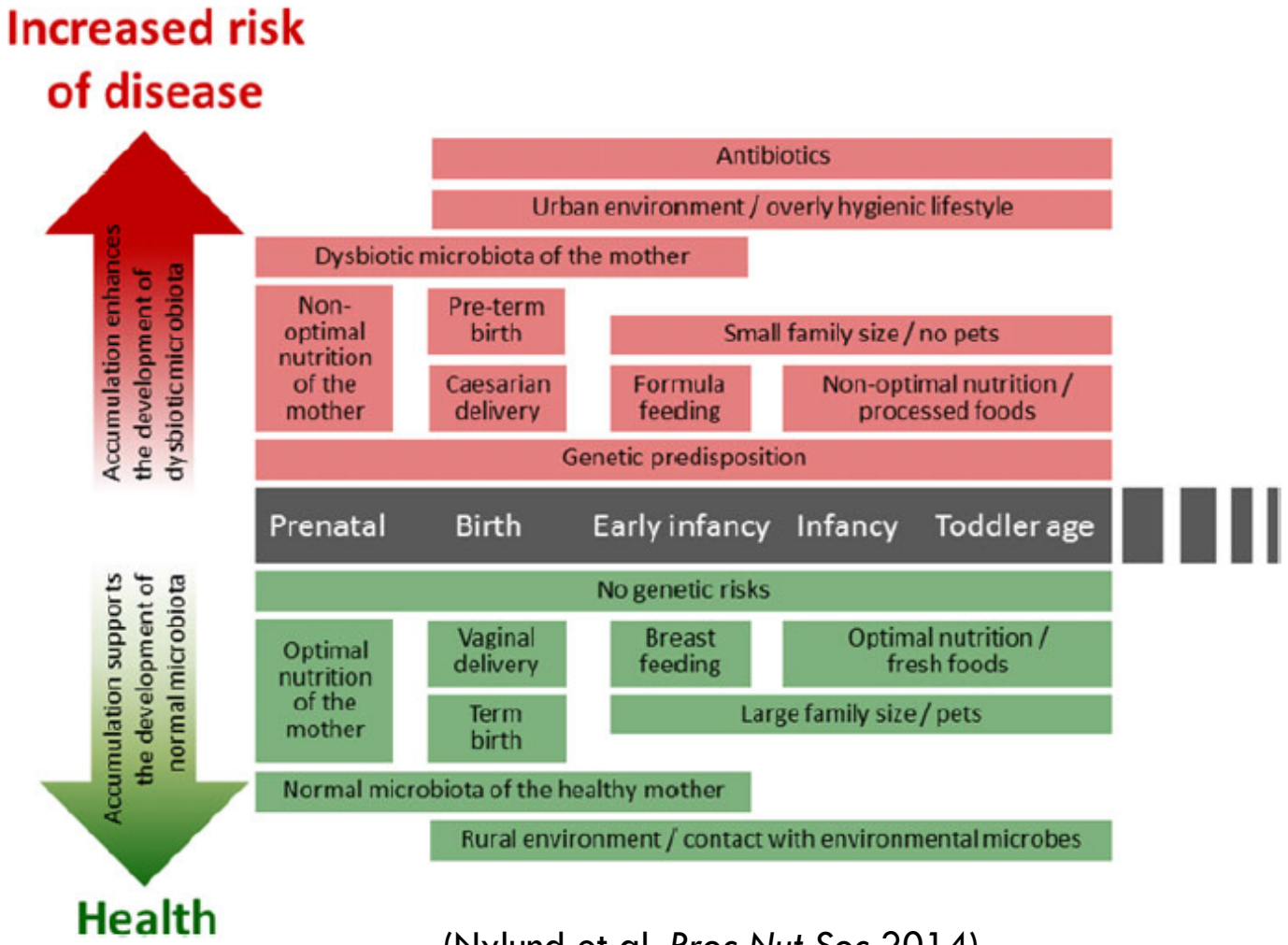


(Tamburini et al. 2017 Nat Med Rev)

Gut Microbiota: Development & Health



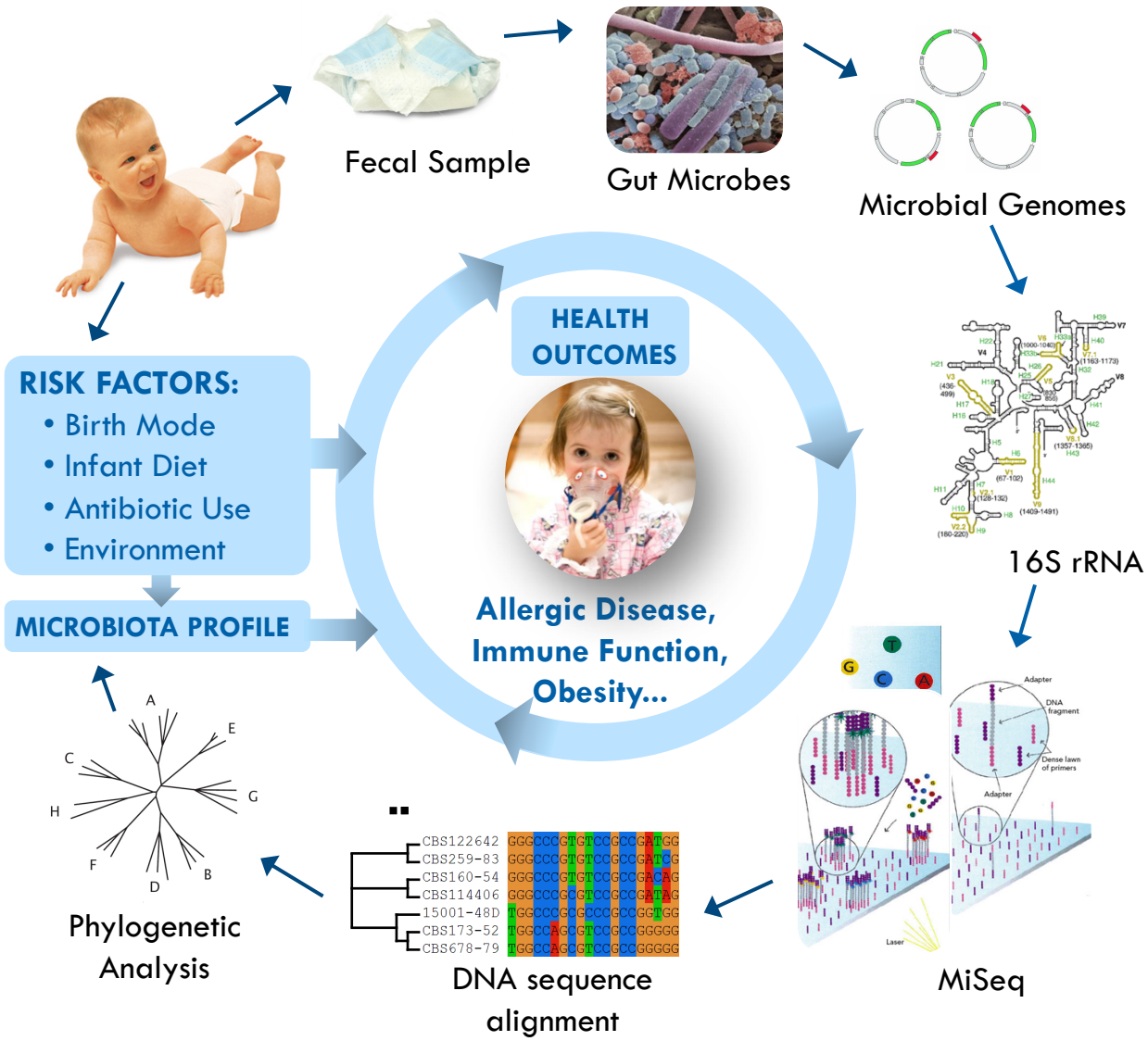
(Putignani et al. Pediatric Research 2014 76:1)



(Nylund et al. *Proc Nut Soc* 2014)

SYMBIOTA
SYNERGY IN MICROBIOTA RESEARCH

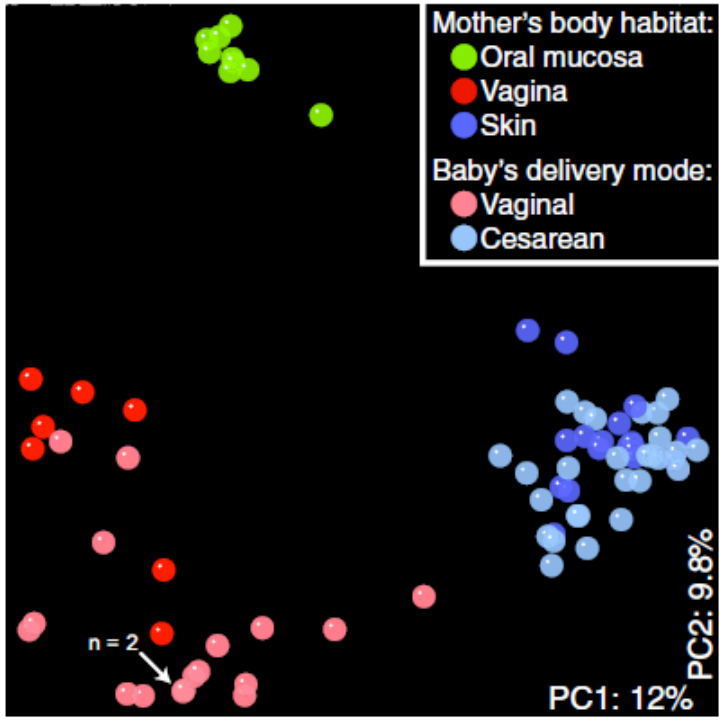
Pls James Scott (Toronto)
Anita Kozyrskyj (Alberta)



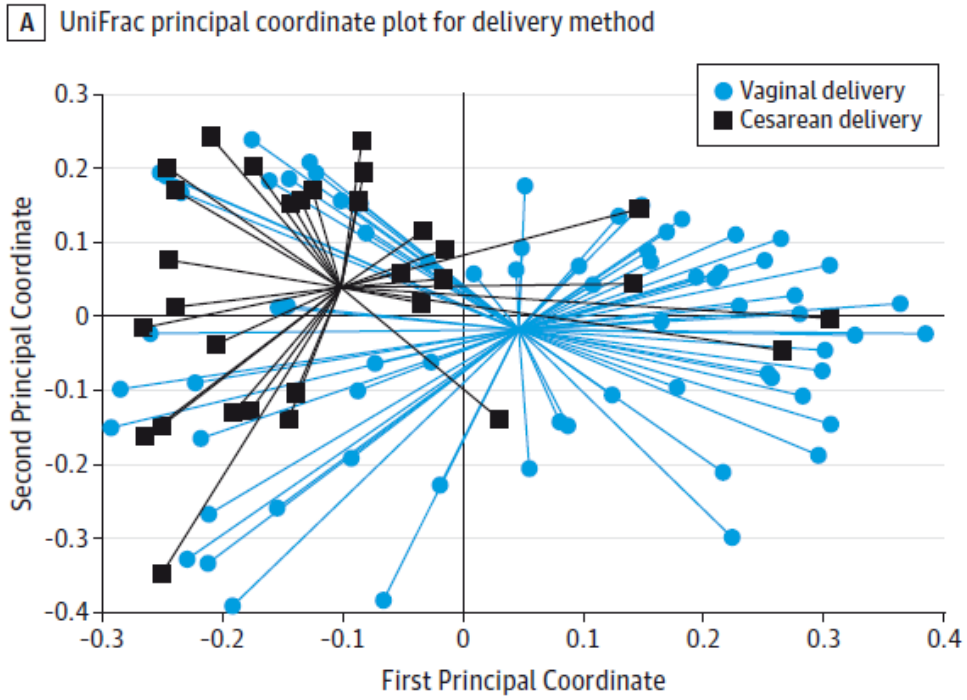
CHILD

Cesarean Section & Microbiota

Vaginally-delivered infants acquire gut microbiota from birth canal,
C-section infants acquire microbiota from skin



(Dominguez-Bello et al. *PNAS* 2010 107:26)



(Madan et al. JAMA Pediatrics 2016)

Perinatal Exposures & Gut Microbiota

CMAJ RESEARCH

Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months

Meghan B. Azad PhD, Theodore Konya MPH, Heather Maughan PhD, David S. Guttman PhD, Catherine J. Field PhD, Radha S. Chari MD, Malcolm R. Sears MB, Allan B. Becker MD, James A. Scott PhD, Anita L. Kozyrskyj PhD, on behalf of the CHILD Study Investigators

See related commentary by Song and colleagues on page 373 and at www.cmaj.ca/lookup/doi/10.1503/cmaj.130147

ABSTRACT

Background: The gut microbiota is essential to human health throughout life, yet the acquisition and development of this microbial community during infancy remains poorly understood. Meanwhile, there is increasing concern over rising rates of cesarean delivery and insufficient exclusive breastfeeding of infants in developed countries. In this article, we characterize the gut microbiota of healthy Canadian infants and describe the influence of cesarean delivery and formula feeding.

Methods: We included a subset of 24 term infants from the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort. Mode of delivery was obtained from medical records, and mothers were asked to report on infant diet and medication use. Fecal samples were collected at 4 months of age, and we characterized the microbiota composition using high-throughput DNA sequencing.

Results: We observed high variability in the profiles of fecal microbiota among the infants. The profiles were generally dominated by Actinobacteria (mainly the genus *Bifidobacterium*) and Firmicutes (with diverse representation from numerous genera). Compared with breastfed infants, formula-fed infants had increased richness of species, with overrepresentation of *Clostridium difficile*. *Escherichia–Shigella* and *Bacteroides* species were underrepresented in infants born by cesarean delivery. Infants born by elective cesarean delivery had particularly low bacterial richness and diversity.

Interpretation: These findings advance our understanding of the gut microbiota in healthy infants. They also provide new evidence for the effects of delivery mode and infant diet as determinants of this essential microbial community in early life.

Competing interests: Allan Becker is an advisory board member for Merck, Novartis and AstraZeneca; his institution has received research grants from Merck and AstraZeneca. No competing interests were declared by the other authors.

This article has been peer reviewed.

Additional CHILD Study Investigators are listed at the end of the article.

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CMAJ 2013. DOI:10.1503/cmaj.121189

DOI: 10.1111/1471-0528.13601
www.bjog.org

BJOG An International Journal of Obstetrics and Gynaecology

Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: a prospective cohort study

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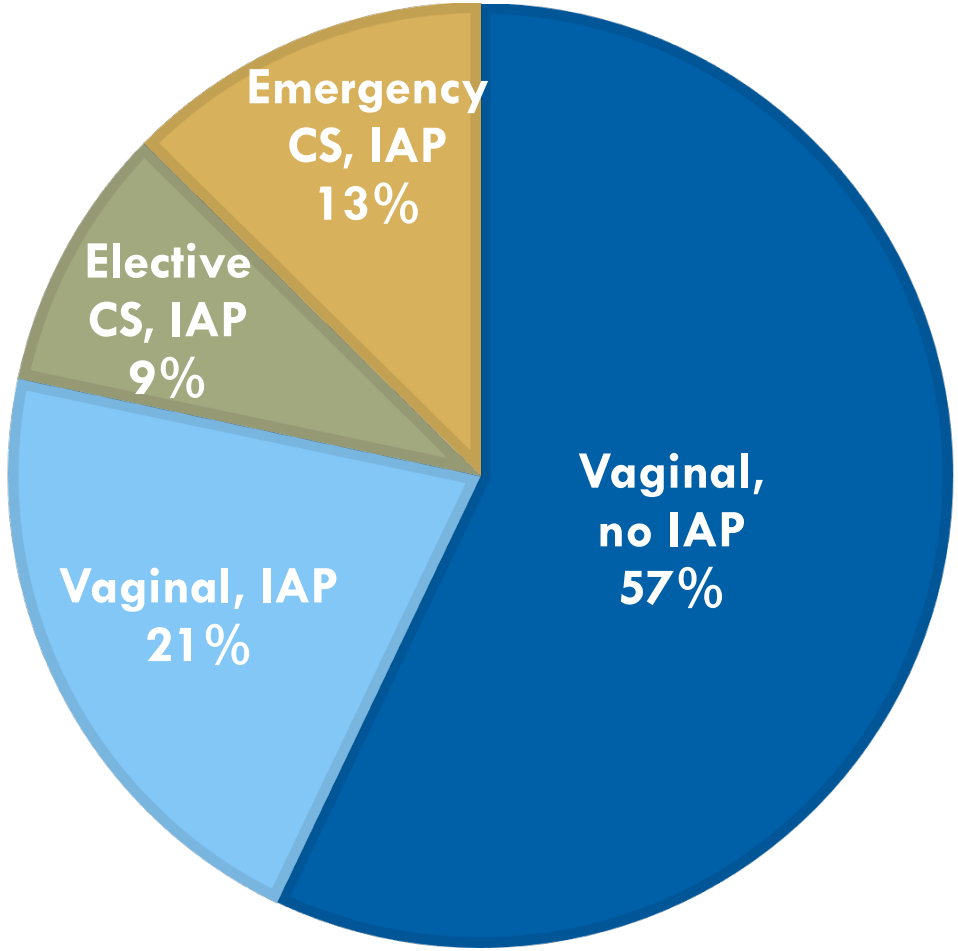
Correspondence: AL Kozyrskyj, PhD, Department of Pediatrics, University of Alberta, 3-527 Edmonton Clinic Health Academy, 11405 – 87th Avenue, Edmonton, AB, Canada T6G IC9. Email kozyrsky@ualberta.ca

Accepted 17 June 2015. Published Online 28 September 2015.

C-Section, Antibiotics & Microbiota



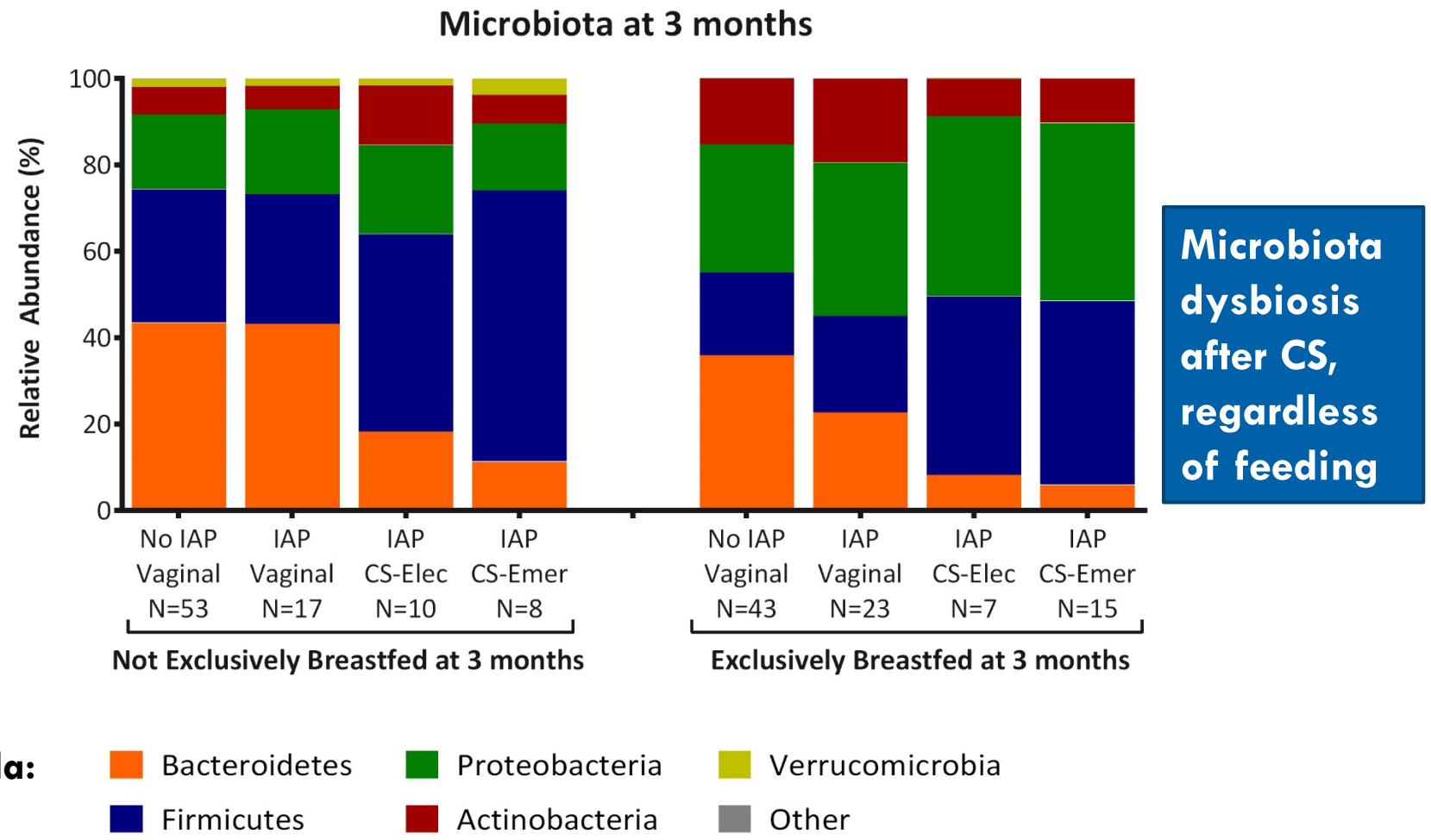
N = 198 mothers from the CHILD Study

Intrapartum Antibiotic Prophylaxis (IAP) administered for:

- ALL Cesarean (CS) deliveries
- 27% of Vaginal deliveries:
  - GBS (76%)
  - PROM (24%)

(Azad et al. BJOG 2015)

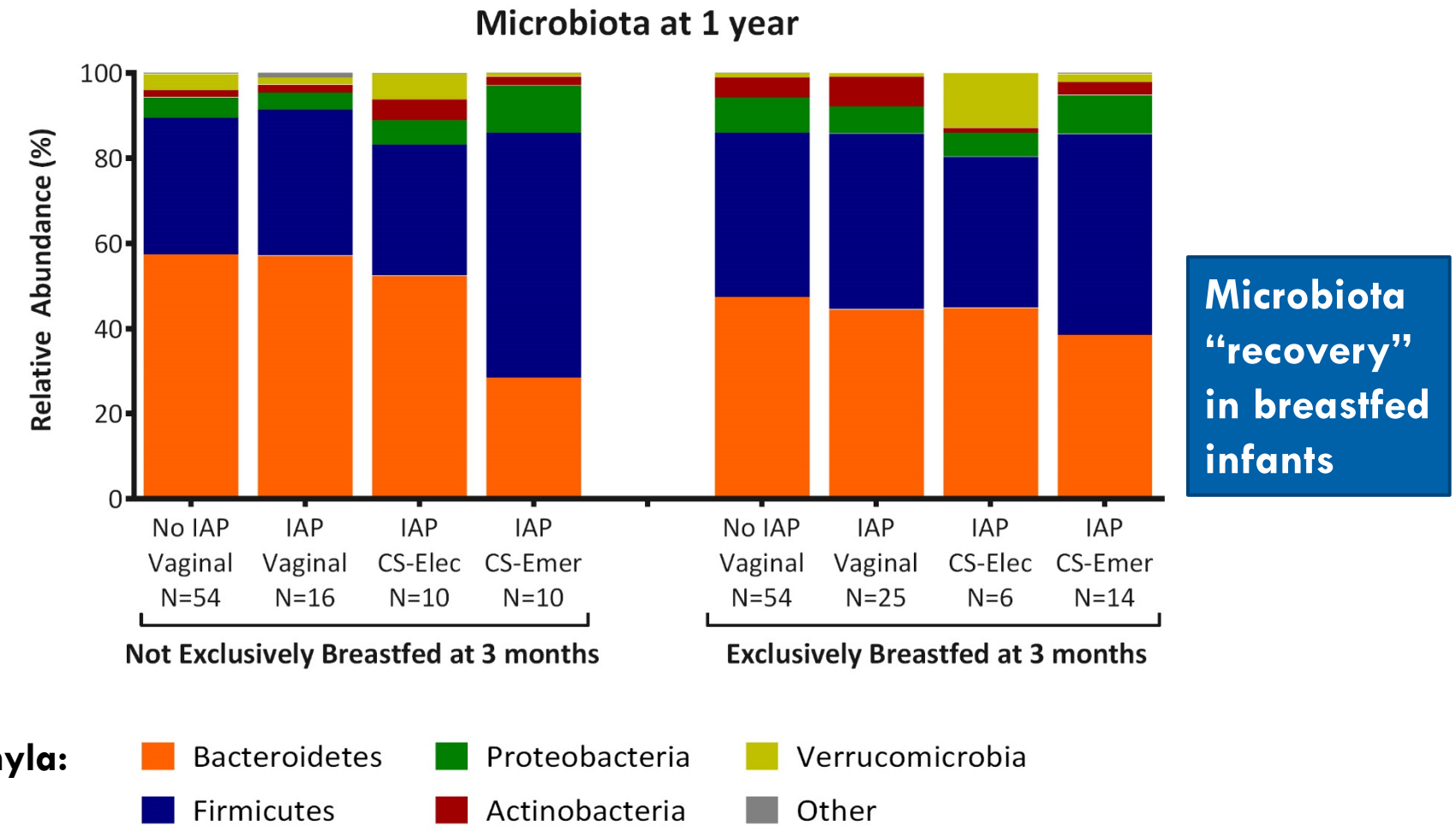
C-Section, Antibiotics, Breastfeeding & Microbiota



Microbiota dysbiosis after CS, regardless of feeding

(Azad et al. BJOG 2015)

C-Section, Antibiotics, Breastfeeding & Microbiota



Microbiota "recovery" in breastfed infants

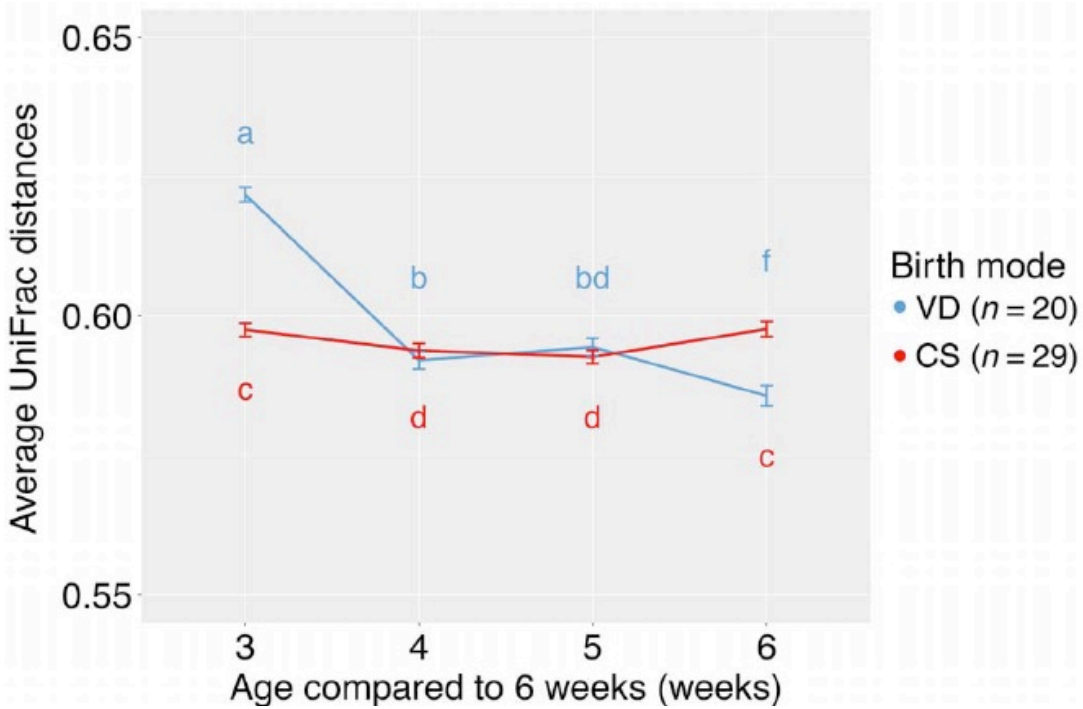
(Azad et al. BJOG 2015)

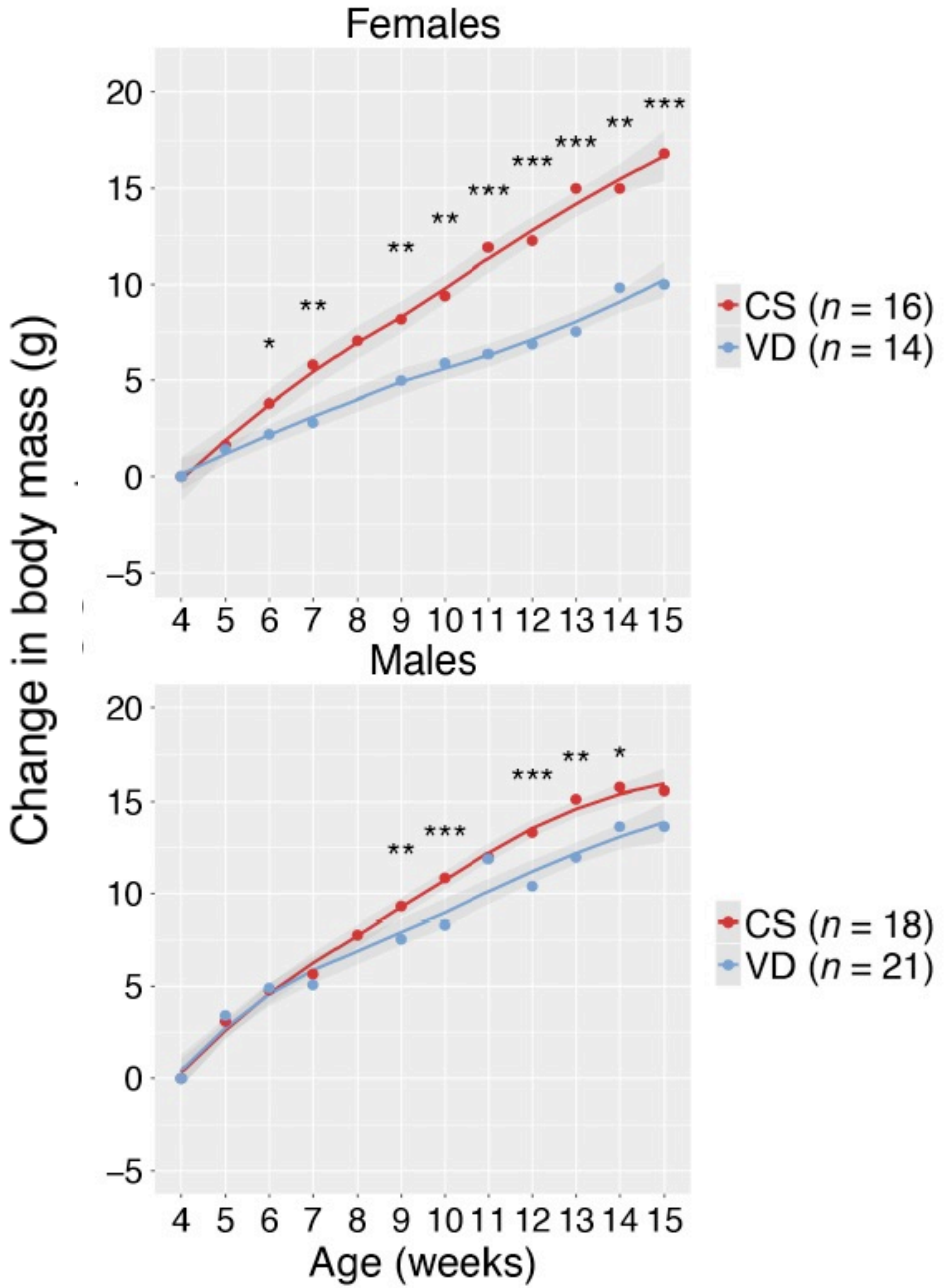
GUT MICROBIOTA

Increased weight gain by C-section: Functional significance of the primordial microbiome

Keith A. Martinez II,[1,2]* Joseph C. Devlin,[1]* Corey R. Lacher,[1] Yue Yin,[1] Yi Cai,[1] Jincheng Wang,[1] Maria G. Dominguez-Bello[1,2]†

Epidemiological evidence supports a direct association between early microbiota impact—including C-section—and obesity. We performed antibiotic-free, fostered C-sections and determined the impact on the early microbiota and body weight during development. Mice in the C-section group gained more body mass after weaning, with a stronger phenotype in females. C-section–born mice lacked the dynamic developmental gut microbiota changes observed in control mice. The results demonstrate a causal relationship between C-section and increased body weight, supporting the involvement of maternal vaginal bacteria in normal metabolic development.





N=142 children (Finland)

ARTICLE NATURE COMMUNICATIONS |

Received 23 Jun 2015 | Accepted 3 Dec 2015 | Published 26 Jan 2016

DOI: 10.1038/ncomms10410 OPEN

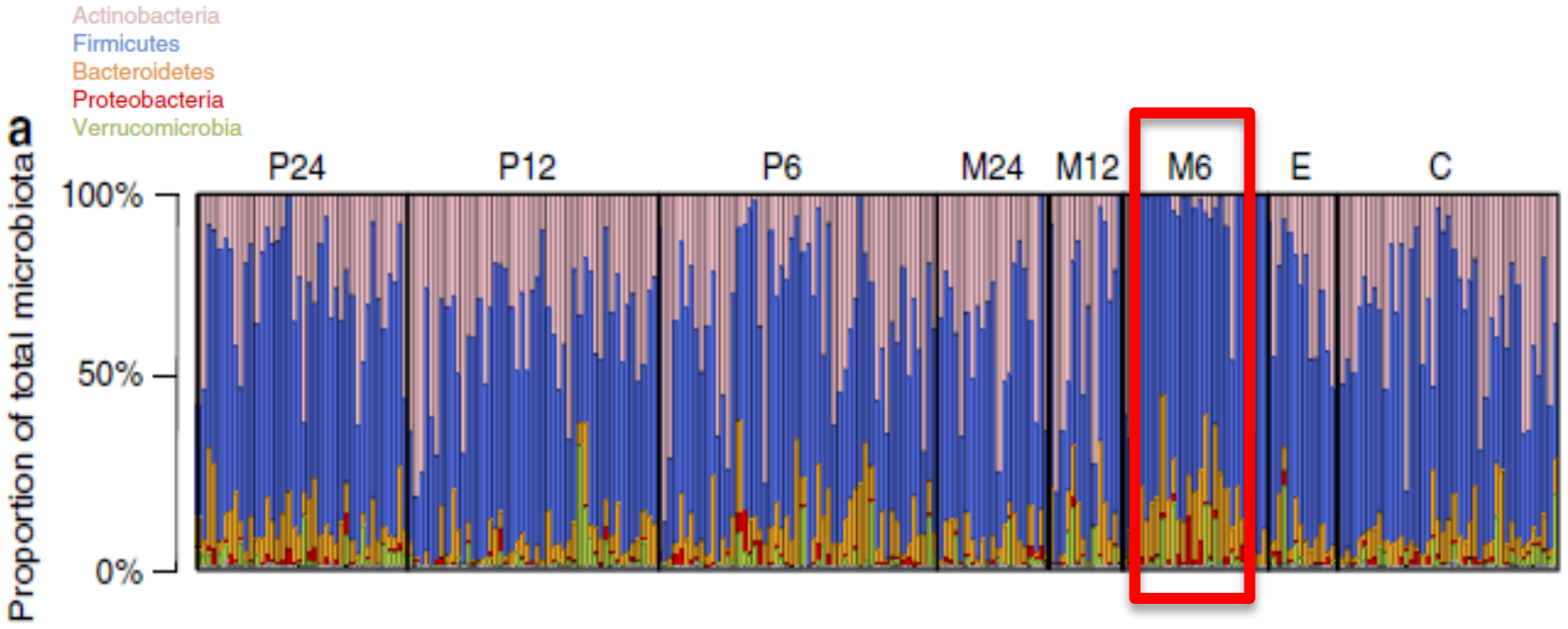
Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children

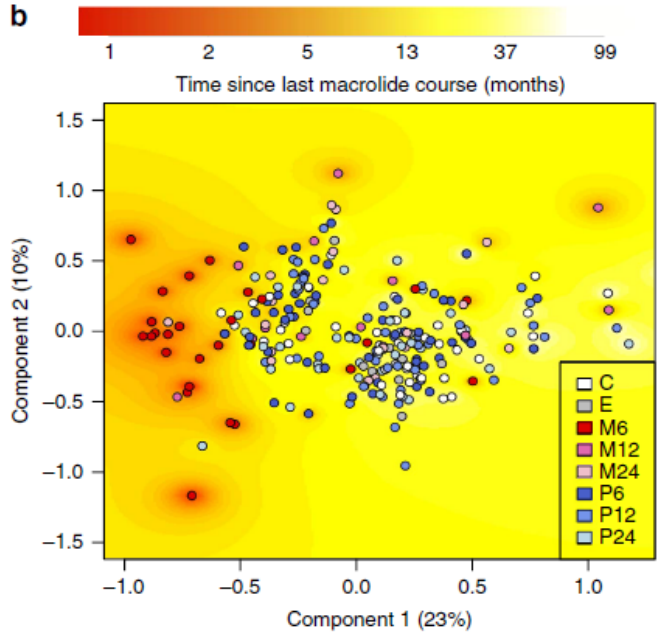
Katri Korpela[1], Anne Salonen[1], Lauri J. Virta[2], Riina A. Kekkonen[3], Kristoffer Forslund[4], Peer Bork[4] & Willem M. de Vos[1,5,6]

Macrolide (M) Antibiotics:

- ↓ Actinobacteria (*Bifidobacteria*)
- ↑ Proteobacteria, Bacteroidetes
- Recovery by 12 months

(No phylum-level effect from Penicillins (P))





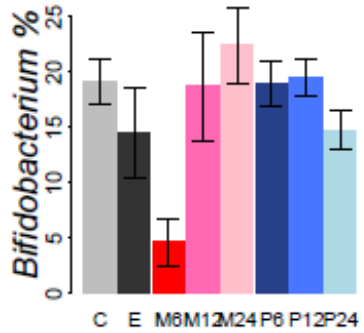


Figure 1 | Microbiota composition in 257 fecal samples as arranged per group. C denotes control group, no antibiotics for the past 2 years and in total <1 course per year of life on average. E denotes early-life exposure group, no antibiotics for the past 2 years and >1 course per year of life on average. M6 denotes macrolide course within 6 months; M12 denotes macrolide course within 6–12 months; M24 denotes macrolide course within 12–24 months. P6, P12 and P24 denote penicillin courses within 6, 6–12 and 12–24 months, respectively. (**a**) Phyla composition. (**b**) Genus-level microbiota composition according to PCoA analysis. The background colour indicates interpolated time since the last macrolide course.

Microbiota "recovery"?

Cell *158*, 705–721, August 14, 2014

Cell

Altering the Intestinal Microbiota during a Critical Developmental Window Has Lasting Metabolic Consequences

Laura M. Cox,[1,2] Shingo Yamanishi,[2] Jiho Sohn,[2] Alexander V. Alekseyenko,[2,3] Jacqueline M. Leung,[1] Ilseung Cho,[2] Sungheon G. Kim,[4] Huilin Li,[5] Zhan Gao,[2] Douglas Mahana,[1] Jorge G. Zárate Rodriguez,[7] Arlin B. Rogers,[6] Nicolas Robine,[8] P'ng Loke,[1] and Martin J. Blaser[1,2,9,*]

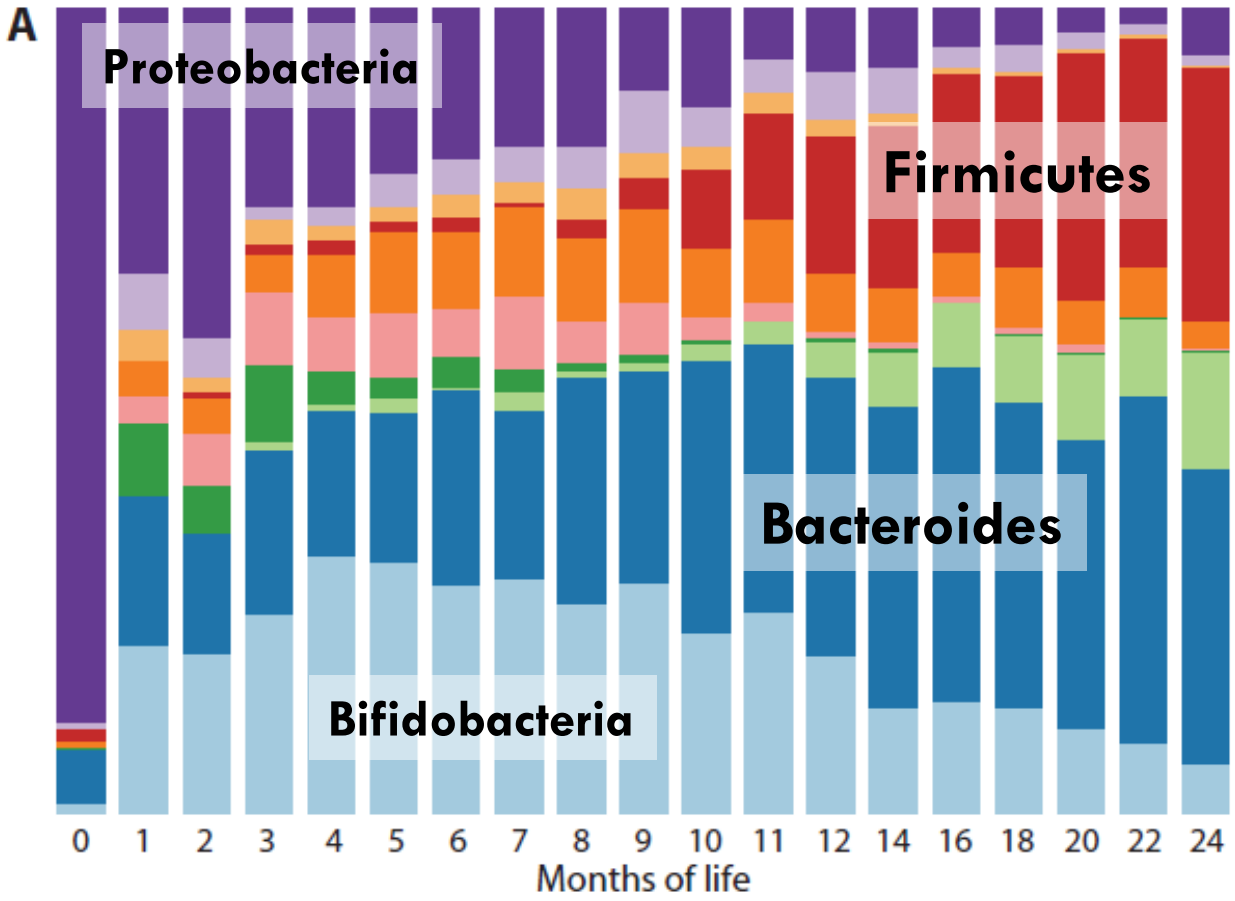
- Antibiotics used to disrupt microbiota in newborn mice
- Microbiota **recovered** after antibiotic exposure, but immune function and adiposity were **permanently** altered

MICROBIOME www.ScienceTranslationalMedicine.org 15 June 2016 Vol 8 Issue 343 343ra82

N=43 infants (USA)

Antibiotics, birth mode, and diet shape microbiome maturation during early life

Nicholas A. Bokulich,[1] Jennifer Chung,[1] Thomas Battaglia,[1] Nora Henderson,[1] Melanie Jay,[1,2] Huilin Li,[3] Arnon D. Lieber,[1] Fen Wu,[1,2] Guillermo I. Perez-Perez,[1,4] Yu Chen,[1,2] William Schweizer,[5] Xuhui Zheng,[4] Monica Contreras,[1] Maria Gloria Dominguez-Bello,[1] Martin J. Blaser[1,4,6]*



Normal development of gut microbiota, birth – 23 months

Actinobacteria|Actinobacteria|Bifidobacteriales|Bifidobacteriaceae|*Bifidobacterium*
Bacteroidetes|Bacteroidia|Bacteroidales|Bacteroidaceae|*Bacteroides*
Firmicutes|Clostridia|Clostridiales
Firmicutes|Clostridia|Clostridiales|Clostridiaceae|
Firmicutes|Clostridia|Clostridiales|Clostridiaceae|*Clostridium*
Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|[*Ruminococcus*]
Firmicutes|Clostridia|Clostridiales|Ruminococcaceae|*Faecalibacterium*
Firmicutes|Clostridia|Clostridiales|Veillonellaceae|*Veillonella*
Firmicutes|Erysipelotrichi|Erysipelotrichales|Erysipelotrichaceae|
Proteobacteria|Gammaproteobacteria|Enterobacteriales|Enterobacteriaceae|

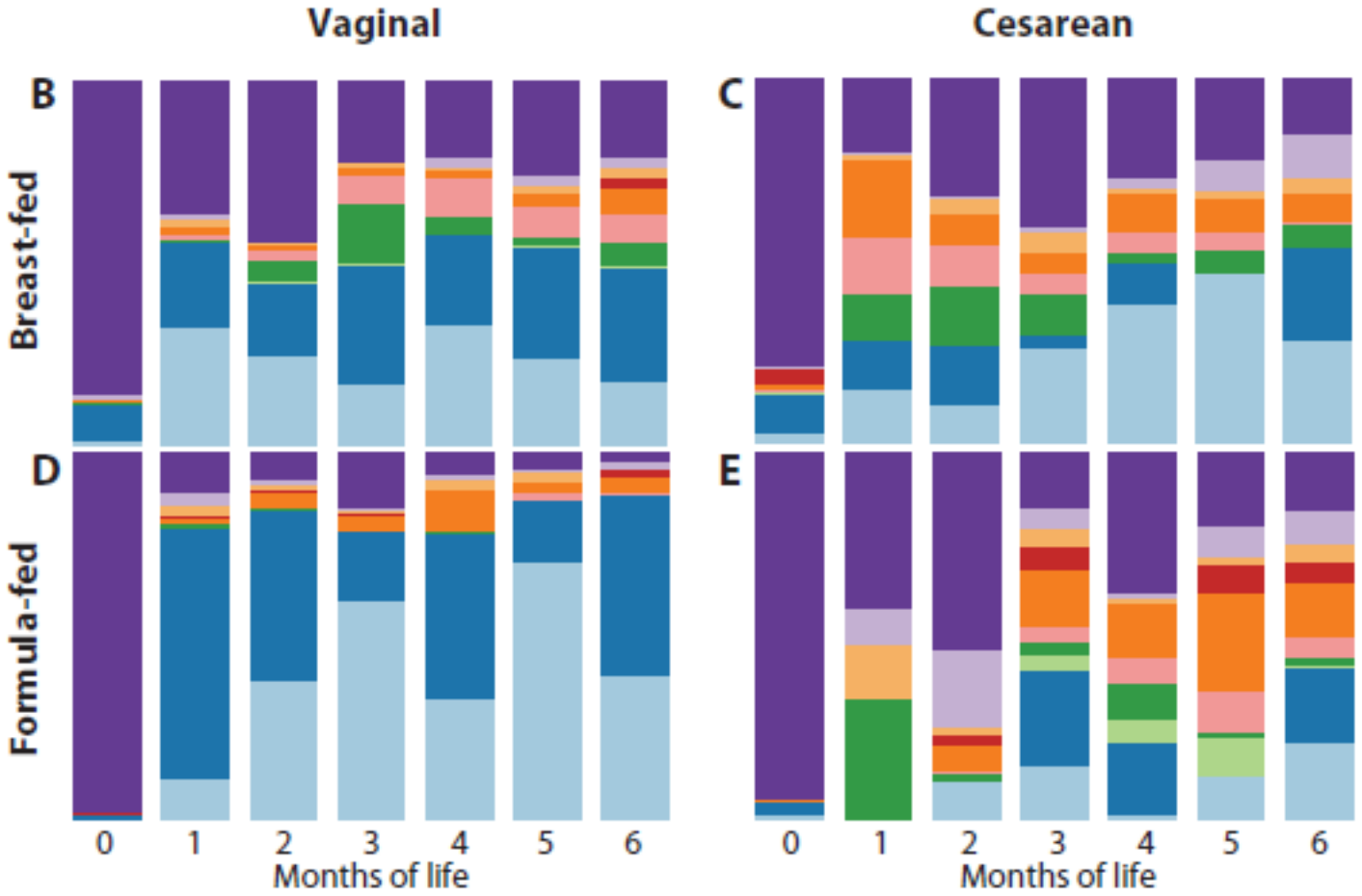
Fig. 1. Microbial and dietary succession viewed over the first 2 years of life. Mean relative abundance (RA) of fecal bacteria at the genus level at each month of life, for taxa with >1% mean RA across all samples. (**A**) All 43 infant subjects during the first 2 years of life. (**B** to **E**) The first 6 months of life for the

MICROBIOME www.ScienceTranslationalMedicine.org 15 June 2016 Vol 8 Issue 343 343ra82

N=43 infants (USA)

Antibiotics, birth mode, and diet shape microbiome maturation during early life

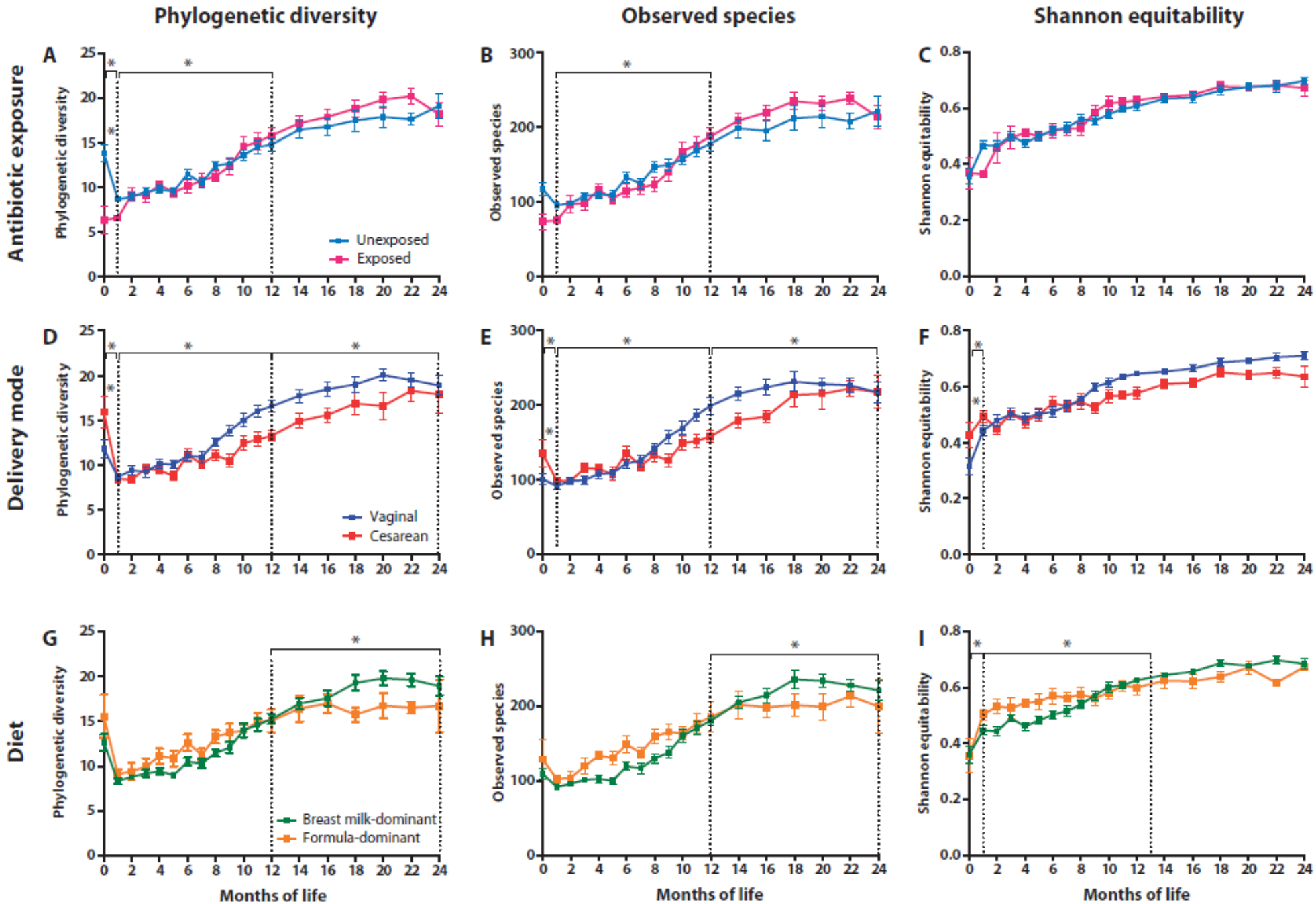
Nicholas A. Bokulich,[1] Jennifer Chung,[1] Thomas Battaglia,[1] Nora Henderson,[1] Melanie Jay,[1,2] Huilin Li,[3] Arnon D. Lieber,[1] Fen Wu,[1,2] Guillermo I. Perez-Perez,[1,4] Yu Chen,[1,2] William Schweizer,[5] Xuhui Zheng,[4] Monica Contreras,[1] Maria Gloria Dominguez-Bello,[1] Martin J. Blaser[1,4,6]*



Differences in microbiome development by birth mode and diet

Actinobacteria|Actinobacteria|Bifidobacteriales|Bifidobacteriaceae|*Bifidobacterium*
Bacteroidetes|Bacteroidia|Bacteroidales|Bacteroidaceae|*Bacteroides*
Firmicutes|Clostridia|Clostridiales
Firmicutes|Clostridia|Clostridiales|Clostridiaceae|
Firmicutes|Clostridia|Clostridiales|Clostridiaceae|*Clostridium*
Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|[*Ruminococcus*]
Firmicutes|Clostridia|Clostridiales|Ruminococcaceae|*Faecalibacterium*
Firmicutes|Clostridia|Clostridiales|Veillonellaceae|*Veillonella*
Firmicutes|Erysipelotrichi|Erysipelotrichales|Erysipelotrichaceae|
Proteobacteria|Gammaproteobacteria|Enterobacteriales|Enterobacteriaceae|

Fig. 1. Microbial and dietary succession viewed over the first 2 years of life. Mean relative abundance (RA) of fecal bacteria at the genus level at each month of life, for taxa with >1% mean RA across all samples. (**A**) All 43 infant subjects during the first 2 years of life. (**B** to **E**) The first 6 months of life for the

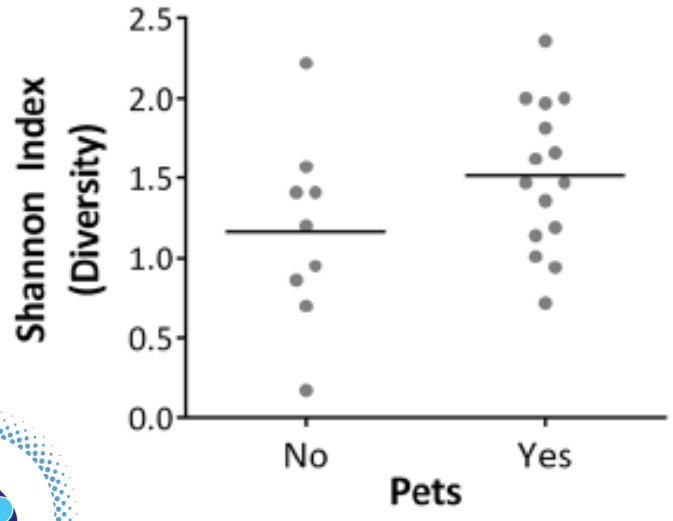


Bokulich et al. 2016

Pets & Microbiota

Infants living with pets have: (Azad et al AACI 2013)

- ↑ gut microbiota diversity
- Different gut microbiota composition



↑ Peptostreptococcaceae (including *C. difficile*)
↑ Clostridiaceae
↑ Veillonaceae (D)
↑ *Coprococcus*
↓ Bifidobacteriaceae (C)
↓ *Eggerthella* (D)
.
.

"Say Hello to the 100 Trillion Bacteria That Make Up Your Microbiome"

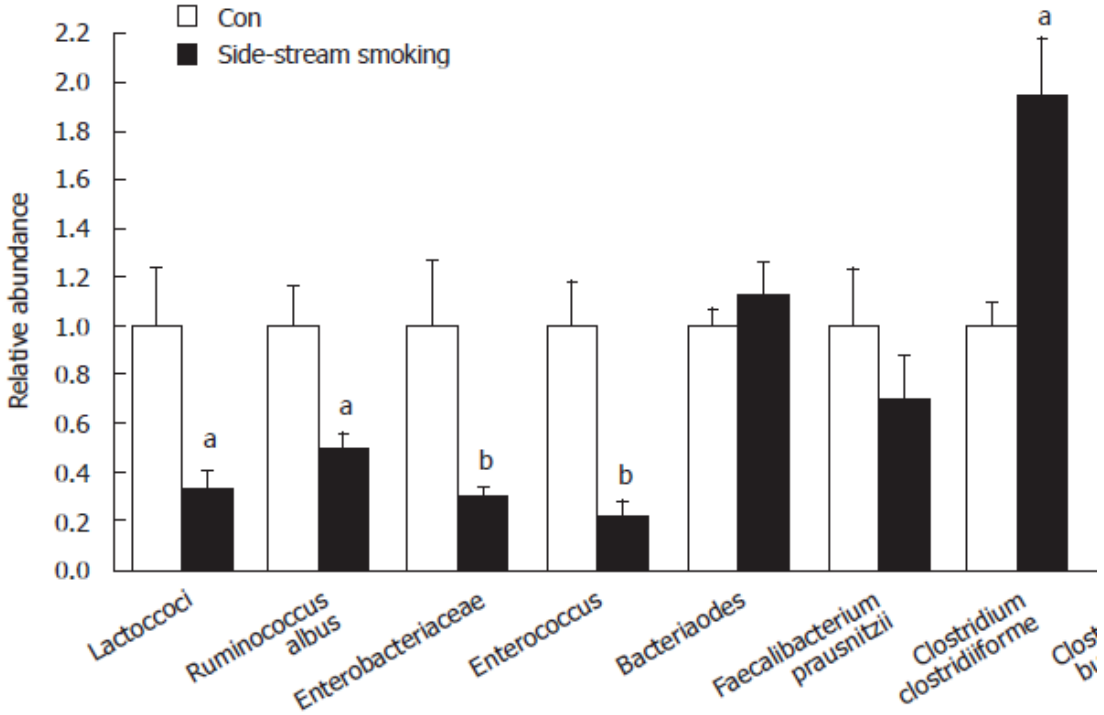
May 15, 2013 ~ NYTimes Magazine
MICHAEL POLLAN

CHILD

Smoking & Microbiota?

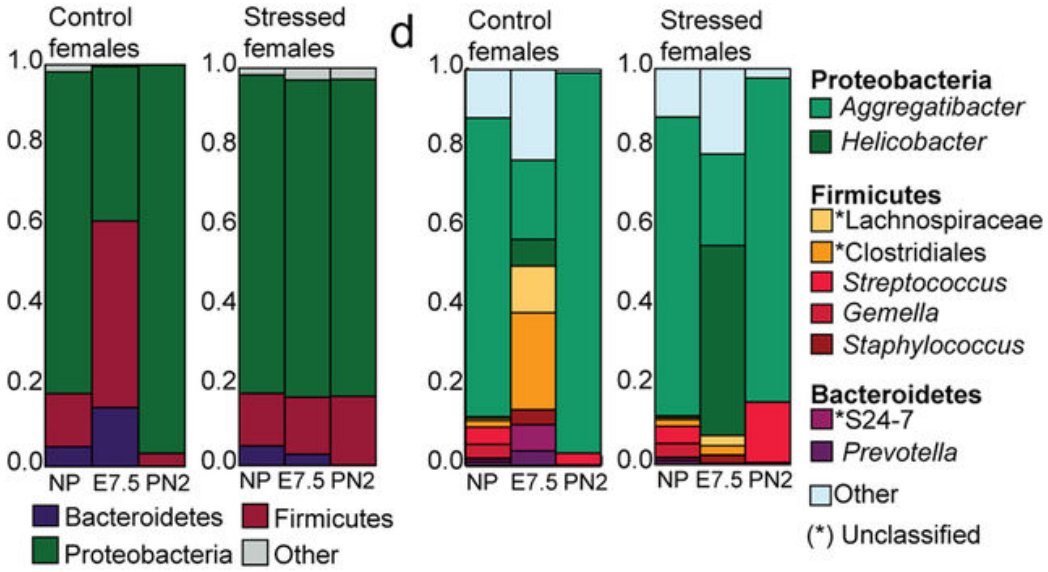
- Second-hand smoke induced significant changes in gut microbiota in mice
  (Wang et al. *World J Gastroenterol* 2012)

- Maternal smoking during pregnancy associated with altered human infant gut microbiota profiles at birth.
  (Gosalbes et al. *Clin Exp Allergy* 2012)



Stress & Microbiota

- No (?) human evidence yet, but…
- Stress during pregnancy in mice alters maternal and offspring microbiome in a sex-specific manner. (Jašarević et al. Sci Rep. 2017)

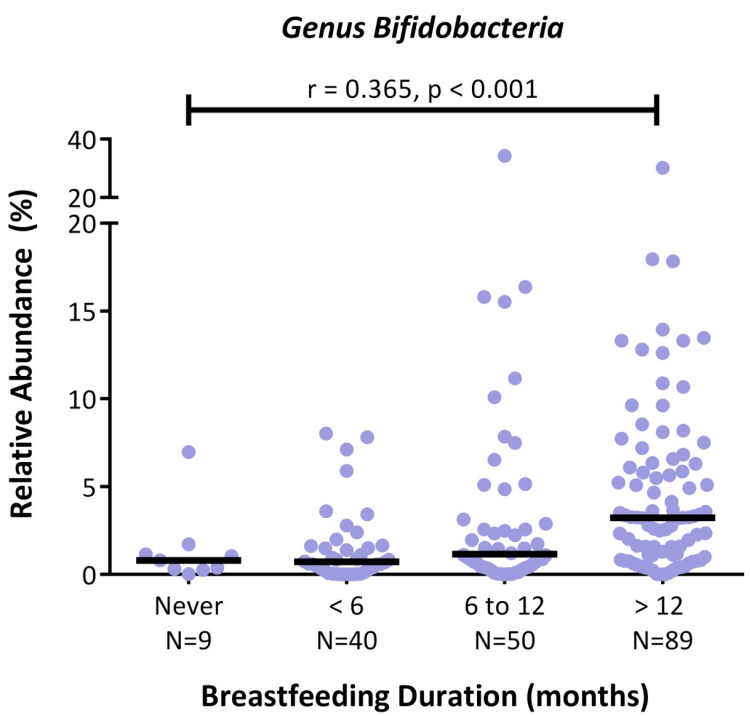


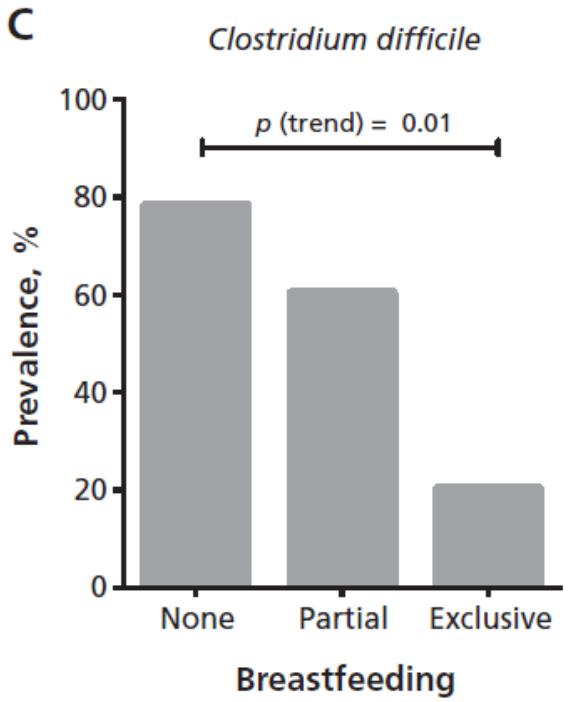
- Gut microbiota composition correlated to grid floor induced stress and behavior in mice. (Bangsgaard Bendtsen et al. PLoS One. 2012 7:10)
- Prenatal stress alters bacterial colonization of the gut in infant monkeys. (Bailey et al. *J Pediatr Gastroenterol Nutr.* 2004 38:4)

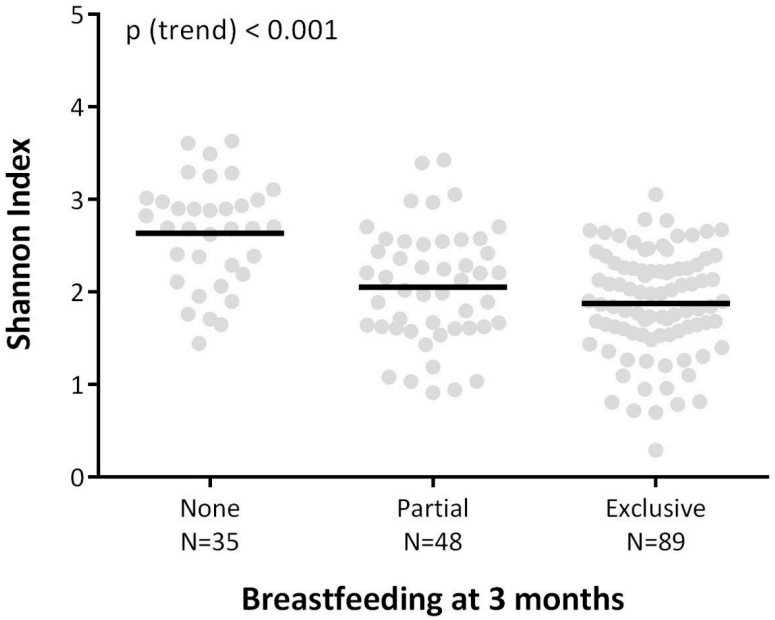
Breastfeeding & Microbiota

Breastfeeding favours:

↑ *Bifidobacteria*, ↓ *Clostridium difficile*, ↓↑ Diversity…







(Azad et al. CMAJ 2013, and BJOG 2015)

DOHaD: Asthma, Allergies & Obesity

- Early **risk** factors:
  - Cesarean section
  - Antibiotics
  - Tobacco smoke
  - Maternal stress
- Early **protective** factors:
  - (Direct) Breastfeeding
  - Pets

Breast milk supplies more than just nutrition for babies.

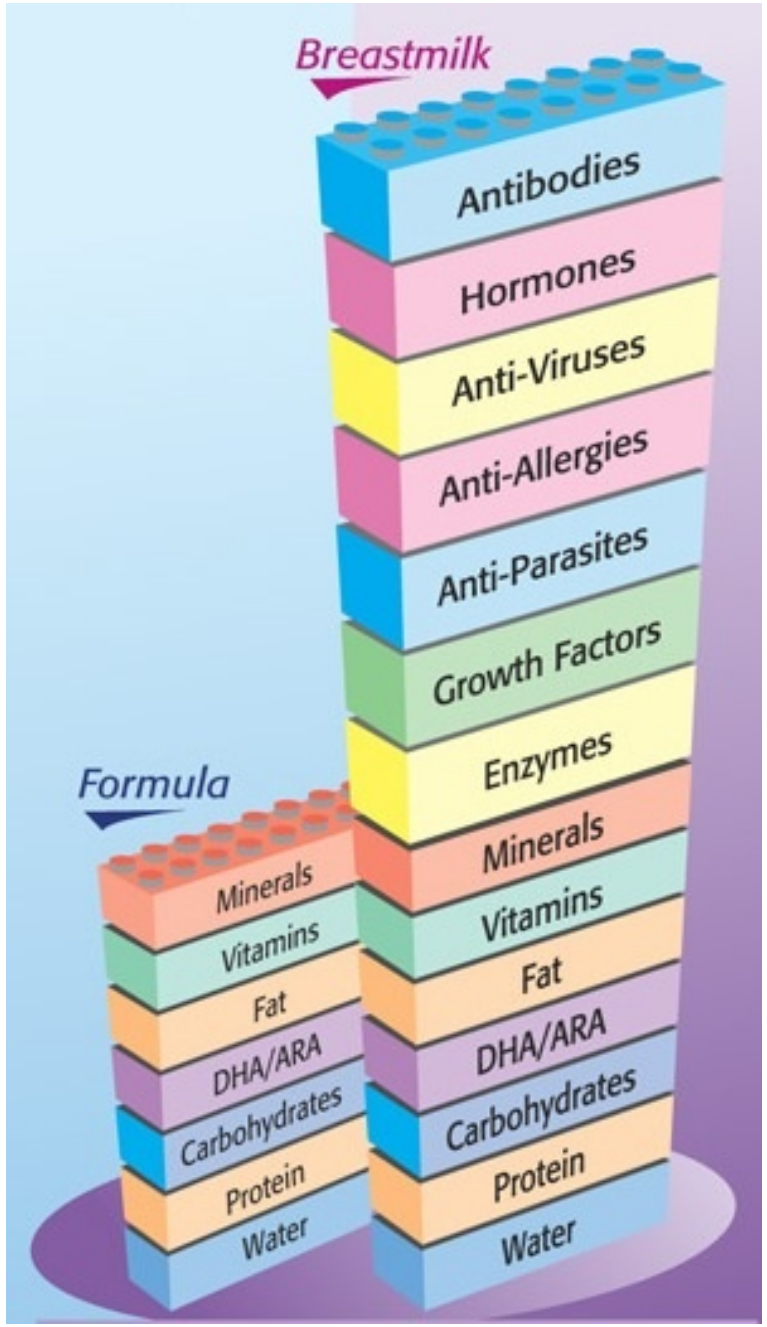
Nature's first functional food

Breast milk feeds helpful microbes, fights harmful ones, provides immunity, and jump-starts a newborn's life

ing explored once again. Sor
have focused on making bet
microbiome fostered by milk
have documented how bre
more than feed a newborn
bacteria. Mother's milk als

"Milk is really a genius fluid that was outrageously understudied. If we can identify components of human breast milk that are important, then we can understand the wisdom of milk–and take advantage of them."

David Mills, UC Davis



DID YOU EVER WONDER WHAT'S IN... ?

BREASTMILK

WATER

CARBOHYDRATES (energy source)
- Lactose
- Oligosaccharides (see below)

CARBOXYLIC ACID
- Alpha hydroxy acid
  - Lactic acid

PROTEINS (building muscles and bones)
- Whey protein
  - Alpha-lactalbumin
    - HAMLET (Human Alpha-lactalbumin Made Lethal to Tumour cells)
  - Lactoferrin
  - Many antimicrobial factors (see below)
- Casein
- Serum albumin

NON-PROTEIN NITROGENS
- Creatine
- Creatinine
- Urea
- Uric acid
- Peptides (see below)
- Amino Acids (the building blocks of proteins)
  - Alanine
  - Arginine
  - Aspartate
  - Cycine
  - Cystine
  - Glutamate
  - Histidine
  - Isoleucine
  - Leucine
  - Lycine
  - Methionine
  - Phenylalanine
  - Proline
  - Serine
  - Taurine
  - Theronine
  - Tryptophan
  - Tyrosine
  - Valine
  - Carnitine (amino acid compound necessary to make use of fatty acids as an energy source)
- Nucleotides (chemical compounds that are the structural units of RNA and DNA)
  - 5'-Adenosine monophosphate (5'-AMP)
  - 3',5'-Cyclic adenosine monophosphate (3',5'-cyclic AMP)
  - 5'-Cytidine monophosphate (5'-CMP)
  - Cytidine diphosphate choline (CDP choline)
  - Guanosine diphosphate (UDP)
  - Guanosine diphosphate - mannose
  - 3'- Uridine monophosphate (3'-UMP)
  - 5'-Uridine monophosphate (5'-UMP)
  - Uridine diphosphate (UDP)
  - Uridine diphosphate hexose (UDPH)
  - Uridine diphosphate-N-acetyl-hexosamine (UDPAH)
  - Uridine diphosphoglucuronic acid (UDPGA)
  - Several more novel nucleotides of the UDP type

FATS
- Triglycerides
  - Long-chain polyunsaturated fatty acids
    - Docosahexaenoic acid (DHA) (important for brain development)
    - Arachidonic acid (AHA) (important for brain development)
    - Linoleic acid
    - Alpha-linolenic acid (ALA)
    - Eicosapentaenoic acid (EPA)
    - Conjugated linoleic acid (Rumenic acid)
  - Free Fatty Acids
  - Monounsaturated fatty acids
    - Oleic acid
    - Palmitoleic acid
    - Heptadecenoic acid
  - Saturated fatty acids
    - Stearic
    - Palmitic acid
    - Lauric acid
    - Myristic acid
- Phospholipids
  - Phosphatidylcholine
  - Phosphatidylethanolamine
  - Phosphatidylinositol
  - Lysophosphatidylcholine
  - Lysophosphatidylethanolamine
  - Plasmalogens
- Sphingolipids
  - Sphingomyelin
  - Gangliosides
    - GM1
    - GM2
    - GM3
  - Glucosylceramide
  - Glycosphingolipids
  - Galactosylceramide
  - Lactosylceramide
  - Globotriaosylceramide (GB3)
  - Globoside (GB4)
- Sterols
  - Squalene
  - Lanosterol
  - Dimethylsterol
  - Methosterol
  - Lathosterol
  - Desmosterol
  - Triacylglycerol
  - Cholesterol
  - 7-dehydrocholesterol
  - Stigma-and campesterol
  - 7-ketocholesterol
  - Sitosterol
  - β-lathosterol
  - Vitamin D metabolites
  - Steroid hormones

VITAMINS
- Vitamin A
- Beta carotene
- Vitamin B6
- Vitamin B8 (Inositol)
- Vitamin B12
- Vitamin C
- Vitamin D
- Vitamin E
- a-tocopherol
- Vitamin K
- Thiamine
- Riboflavin
- Niacin
- Folic acid
- Pantothenic acid
- Biotin

MINERALS
- Calcium
- Sodium
- Potassium
- Iron
- Zinc
- Chloride
- Phosphorus
- Magnesium
- Copper
- Manganese
- Iodine
- Selenium
- Choline
- Sulpher
- Chromium
- Cobalt
- Fluorine
- Nickel

METAL
- Molybdenum (essential element in many enzymes)

GROWTH FACTORS (aid in the maturation of the intestinal lining)
- Cytokines
  - Interleukin-1β (IL-1β)
  - IL-2
  - IL-4
  - IL-6

PEPTIDES (combinations of amino acids)
- HMGF I (Human growth factor)
- HMGF II
- HMGF III
- Cholecystokinin (CCK)
- β-endorphins
- Parathyroid hormone (PTH)
- Parathyroid hormone-related peptide (PTHrP)
- β-defensin-1
- Calcitonin
- Gastrin
- Motilin
- Bombesin (gastric releasing peptide, also known as neuromedin B)
- Neurotensin
- Somatostatin

HORMONES (chemical messengers that carry signals from one cell, or group of cells, to another via the blood)
- Cortisol
- Triiodothyronine (T3)
- Thyroxine (T4)
- Thyroid stimulating hormone (TSH) (also known as thyrotropin)
- Thyroid releasing hormone (TRH)
- Prolactin
- Oxytocin
- Insulin
- Corticosterone
- Thrombopoietin
- Gonadotropin-releasing hormone (GnRH)
- GRH
- Leptin (aids in regulation of food intake)
- Ghrelin (aids in regulation of food intake)
- Adiponectin
- Feedback inhibitor of lactation (FIL)
- Eicosanoids
  - Prostaglandins (enzymatically derived from fatty acids)
    - PG-E1
    - PG-E2
    - PG-F2
  - Leukotrienes
  - Thromboxanes
  - Prostacyclins

ANTIPROTEASES (thought to bind themselves to macromolecules such as enzymes and as a result prevent allergic and anaphylactic reactions)
- a-1-antitrypsin
- a-1-antichymotrypsin

ANTIMICROBIAL FACTORS (are used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses.)
- Leukocytes (white blood cells)
  - Phagocytes
    - Basophils
    - Neutrophils
    - Eosinophils
  - Macrophages
  - Lymphocytes
    - B lymphocytes (also known as B cells)
    - T lymphocytes (also known as C cells)
- sIgA (Secretory immunoglobulin A) (the most important antiinfective factor)
- IgA2
- IgG
- IgD
- IgM
- IgE
- Complement C1
- Complement C2
- Complement C3
- Complement C4
- Complement C5
- Complement C6
- Complement C7
- Complement C8
- Complement C9
- Glycoproteins
  - Mucins (attaches to bacteria and viruses to prevent them from clinging to mucousal tissues)
  - Lactadherin
- Alpha-lactoglobulin
- Alpha-2 macroglobulin
- Lewis antigens
- Ribonuclease
- Haemagglutinin inhibitors
- Bifidus Factor (increases growth of Lactobacillus bifidus - which is a good bacteria)
- Lactoferrin (binds to iron which prevents harmful bacteria from using the iron to grow)

FORMULA

WATER

CARBOHYDRATES
- Lactose
- Corn maltodextrin

PROTEIN
- Partially hydrolyzed reduced minerals whey protein concentrate (from cow's milk)

FATS
- Palm olein
- Soybean oil
- Coconut oil
- High oleic safflower oil (or sunflower oil)
- M. alpina oil (Fungal DHA)
- C.cohnii oil (Algal ARA)

MINERALS
- Potassium citrate
- Potassium phosphate
- Calcium chloride
- Tricalcium phosphate
- Sodium citrate
- Magnesium chloride
- Ferrous sulphate
- Zinc sulphate
- Sodium chloride
- Copper sulphate
- Potassium iodide
- Manganese sulphate
- Sodium selenate

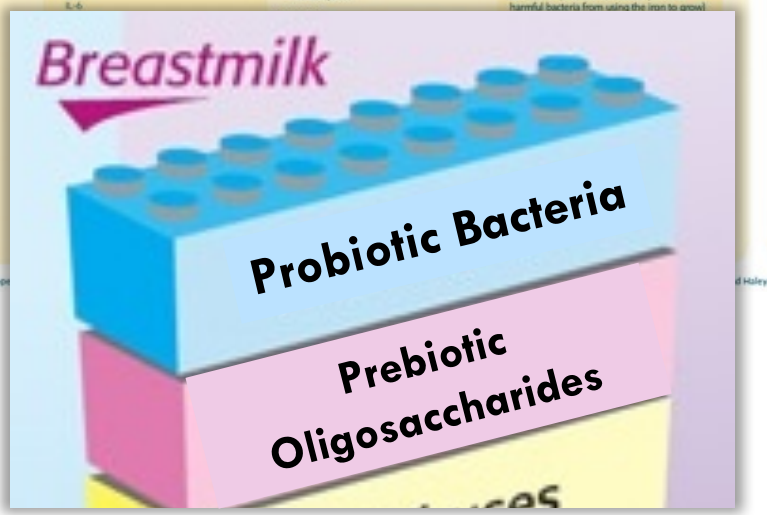
VITAMINS
- Sodium ascorbate
- Inositol
- Choline bitartrate
- Alpha-tocopheryl acetate
- Niacinamide
- Calcium pantothenate
- Riboflavin
- Vitamin A acetate
- Pyridoxine hydrochloride
- Thiamine mononitrate
- Folic acid
- Phylloquinone
- Biotin
- Vitamin D3
- Vitamin B12

ENZYME
- Trypsin

AMINO ACID
- Taurine
- L-Carnitine (a combination of two different amino acids)

NUCLEOTIDES
- Cytidine 5-monophosphate
- Disodium uridine 5-monophosphate
- Adenosine 5-monophosphate
- Disodium guanosine 5-monophosphate
- Soy Lecithin

PLUS:



MICROBIOTA

Mother's littlest helpers

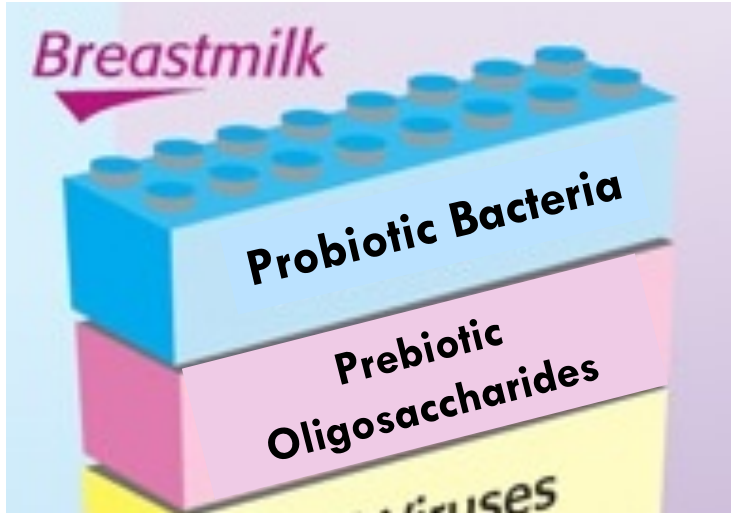
Breastmilk nourishes the microbes colonizing the neonatal intestinal tract

By **Katie Hinde**[1] *and* **Zachery T. Lewis**[2]

Commensal bacteria underlie, in part, our nutritional status, immune function, and psychological well-being. The trillions of beneficial microbes within our intestinal tract convert dietary nutrients, inhibit pathogen colonization, regulate immune processes, and produce neural signals (*1*, *2*). Advances in our understanding of the importance of microbes have motivated the commercial development of products intended to boost "good" commensals and confer health benefits. Probiotic dietary supplements contain live beneficial microbes hoped to subsequently colonize the gut. Prebiotic nutrients are thought to enhance good gastrointestinal microflora by preferentially nourishing beneficial microbes. Even "psychobiotics" are being explored to ameliorate symptoms of psychiatric illness. These live organisms influence the brain through metabolites and neuroactive compounds in rodent models and preliminary human studies (*3*). How to most effectively be the landscape architects of our microbial community, however, often remains unclear. An opportunity to gain insights into how natural selection has shaped the coevolution of hosts and microbes can be found in mammalian mother-infant dyads, as our microbiota are ecologically engineered by the intra-oral vacuum dynamics of suckling (*6*), or via the more speculative translocation to milk through a gut-mammary route (*5*, *7*).

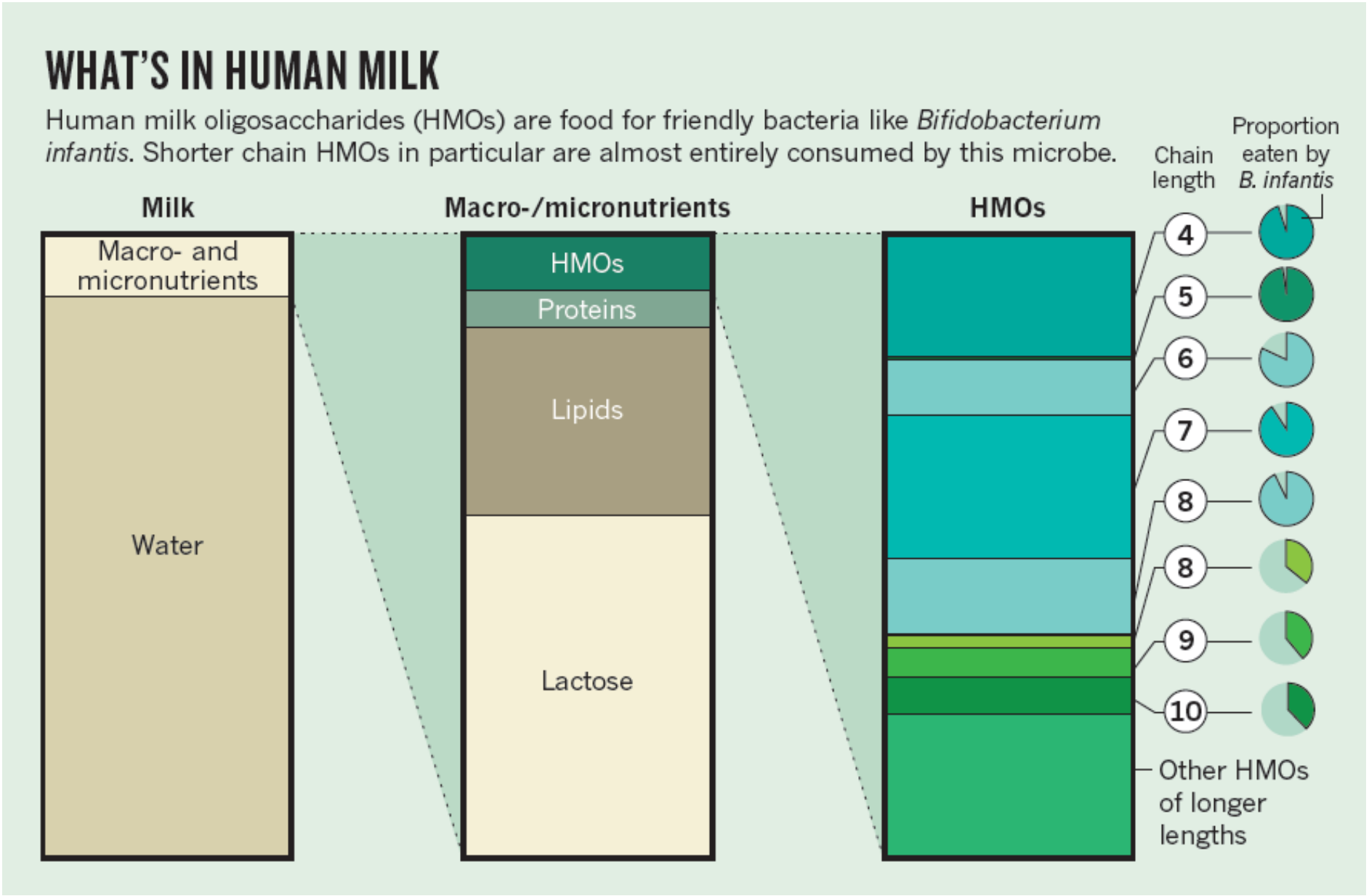
Once breastmilk is in the intestinal environment of an infant, intense microbial competition exists for both space and nutrients. The major available carbon source, human milk glycans, are complex oligosaccharide and glycoconjugate compounds that typically pass undigested from the infant stomach because eutherian mammals (those with a placenta) lack enzymes to cleave them (*8*, *9*). Investigations of the structure of milk oligosaccharides reveal that human milk has a greater diversity (>200 isomers), more complexity, and higher abundance than the milk of other primates, including all of the great apes (*4*, *8*). Importantly, certain oligosaccharides that dominate human milk, but are absent or rare in other primates, are the preferred food of *Bifidobacterium*, the most prevalent microbial clade in the healthy infant gut (*8*).

> ***"...our microbiota are ecologically engineered by mothers and breastmilk."***



Probiotics:
Live beneficial bacteria

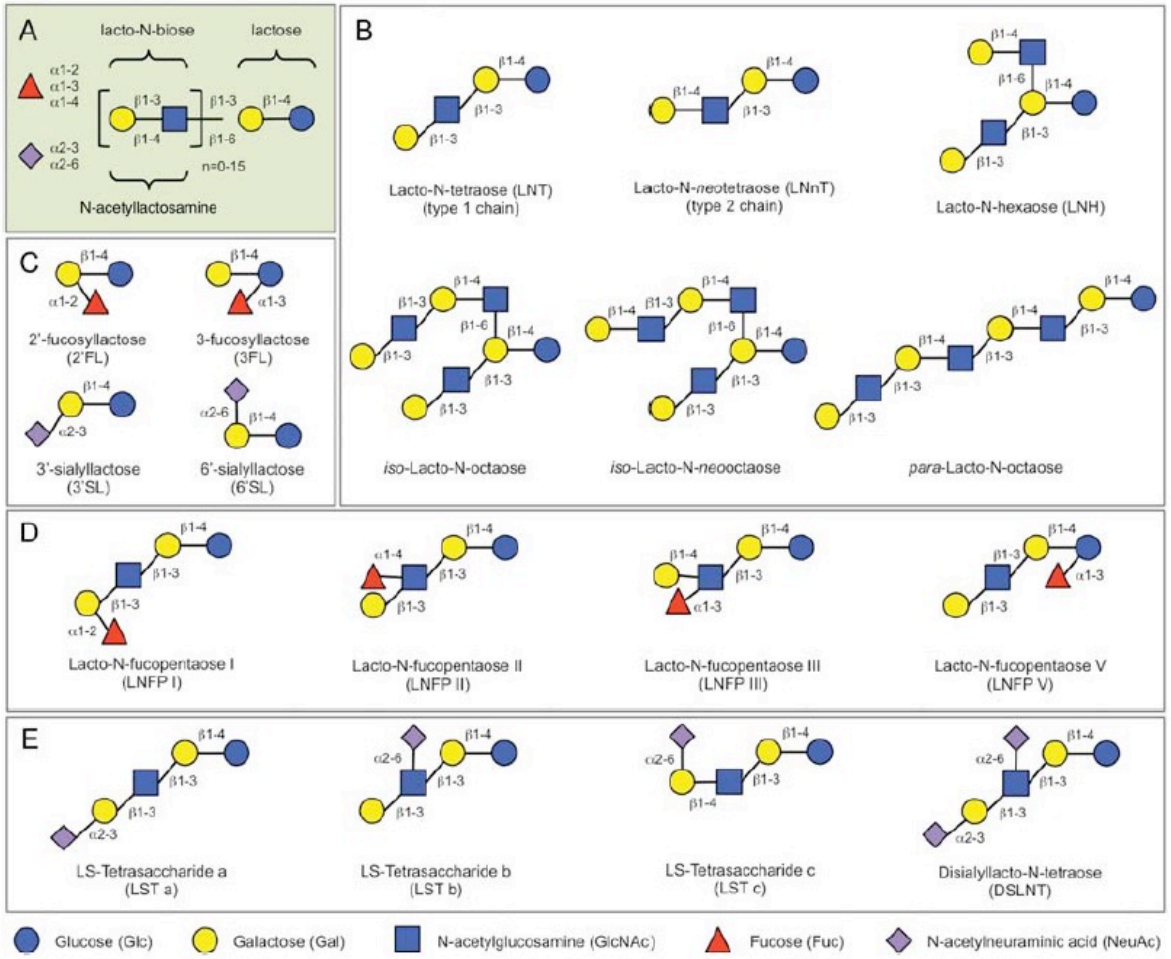
Prebiotics:
Non-digestible carbohydrates that select for beneficial bacteria



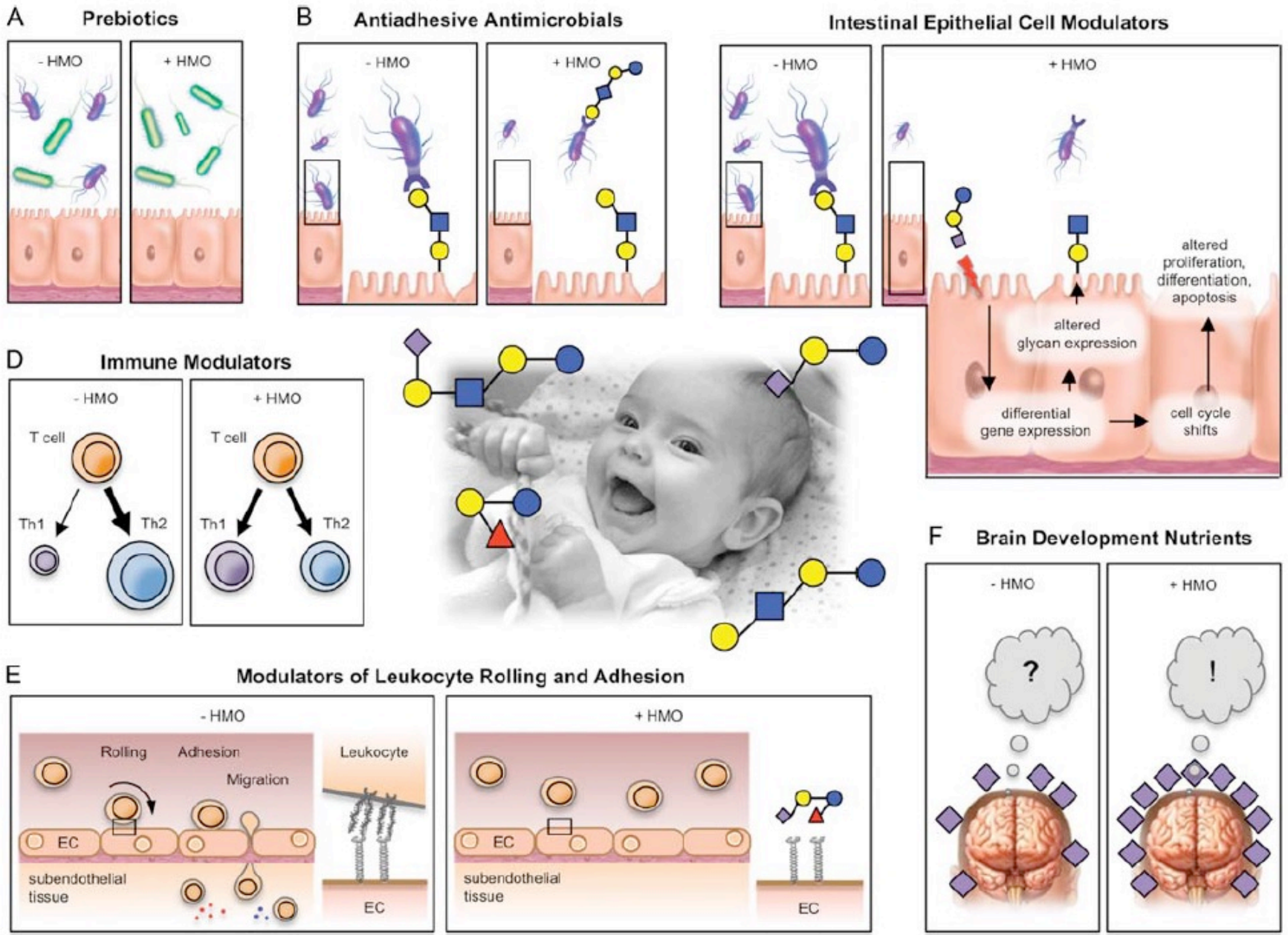
(Petherick *Nature* 2010)

Human Milk Oligosaccharides (HMOs)

- Non-digestible carbohydrates
- Structurally diverse
  Cows: ~40 vs. Humans: >100
- Highly variable between mothers
- Small studies (N<50):
  - Possible associations with HIV transmission, allergy, infant adiposity
  - Maternal determinants (besides genetics) **unknown**



(Bode Glycobiology 2012 –"Every baby needs a sugar mama")



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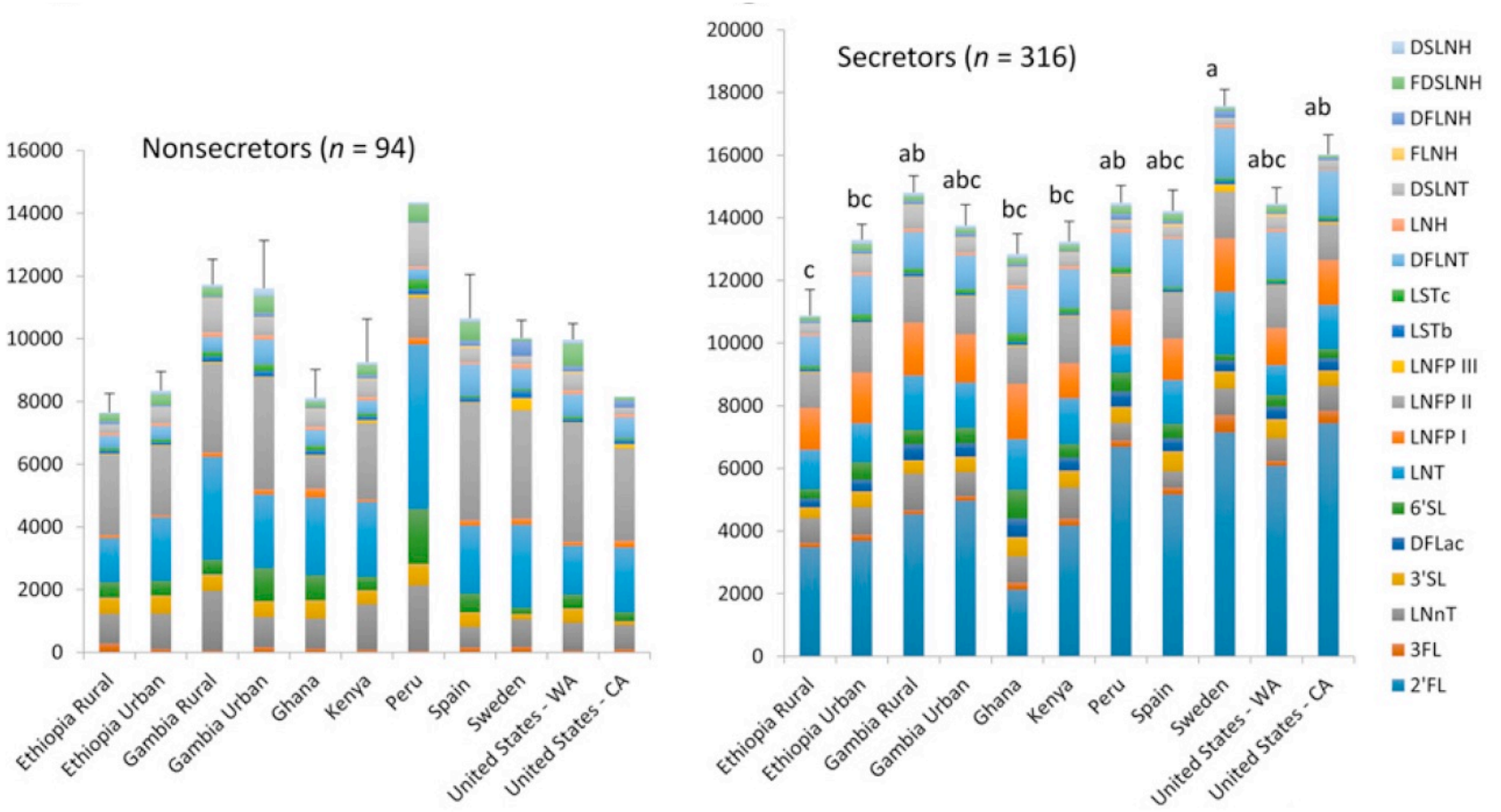
Human Milk Oligosaccharides (HMOs)

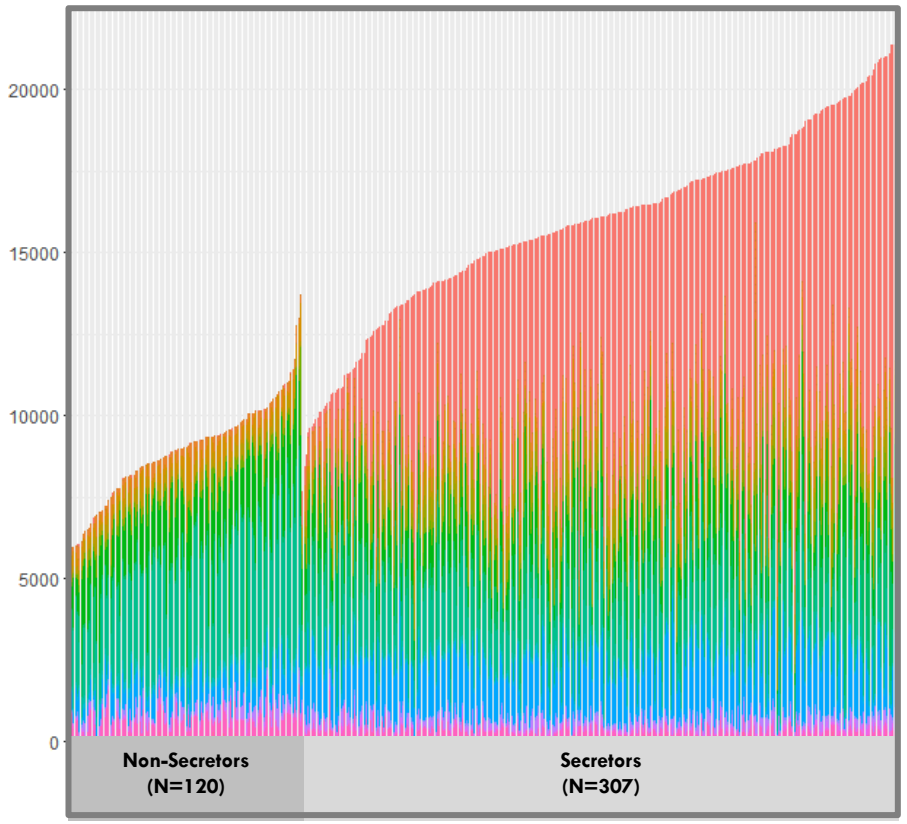


N=410 mothers (9 countries)

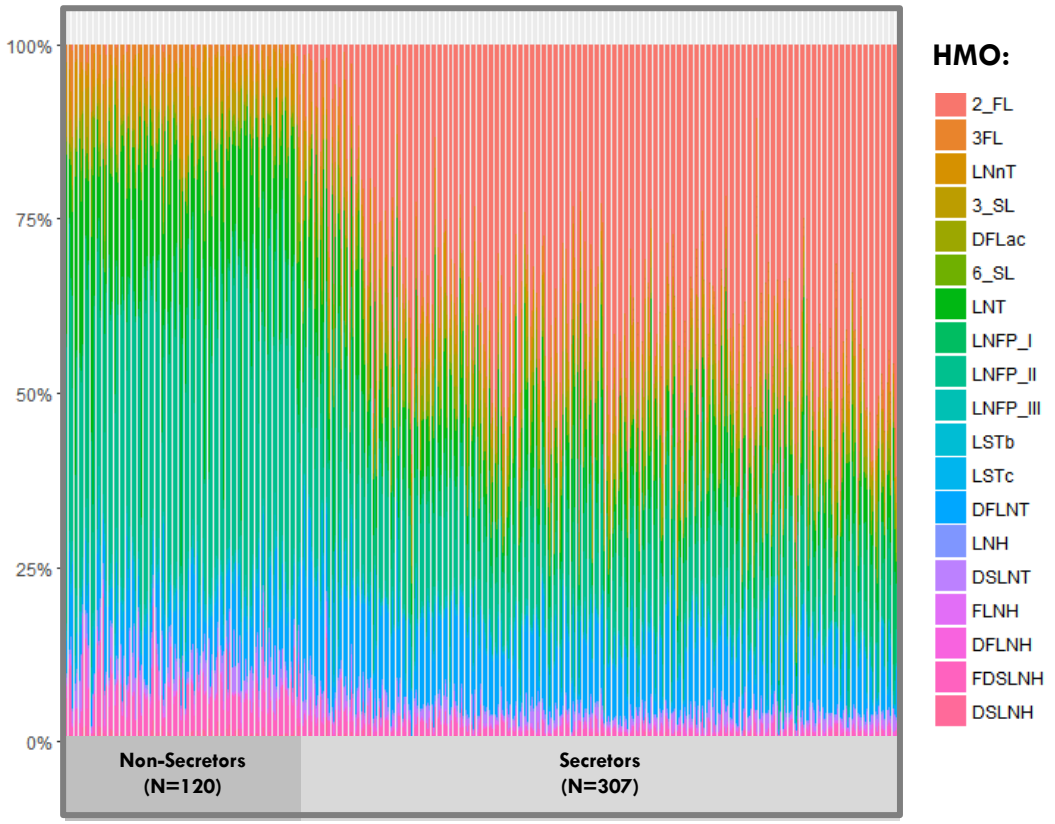
(McGuire et al. AJCN 2017)

HMOs in the CHILD Cohort

Absolute HMO Concentration (nmol/mL)



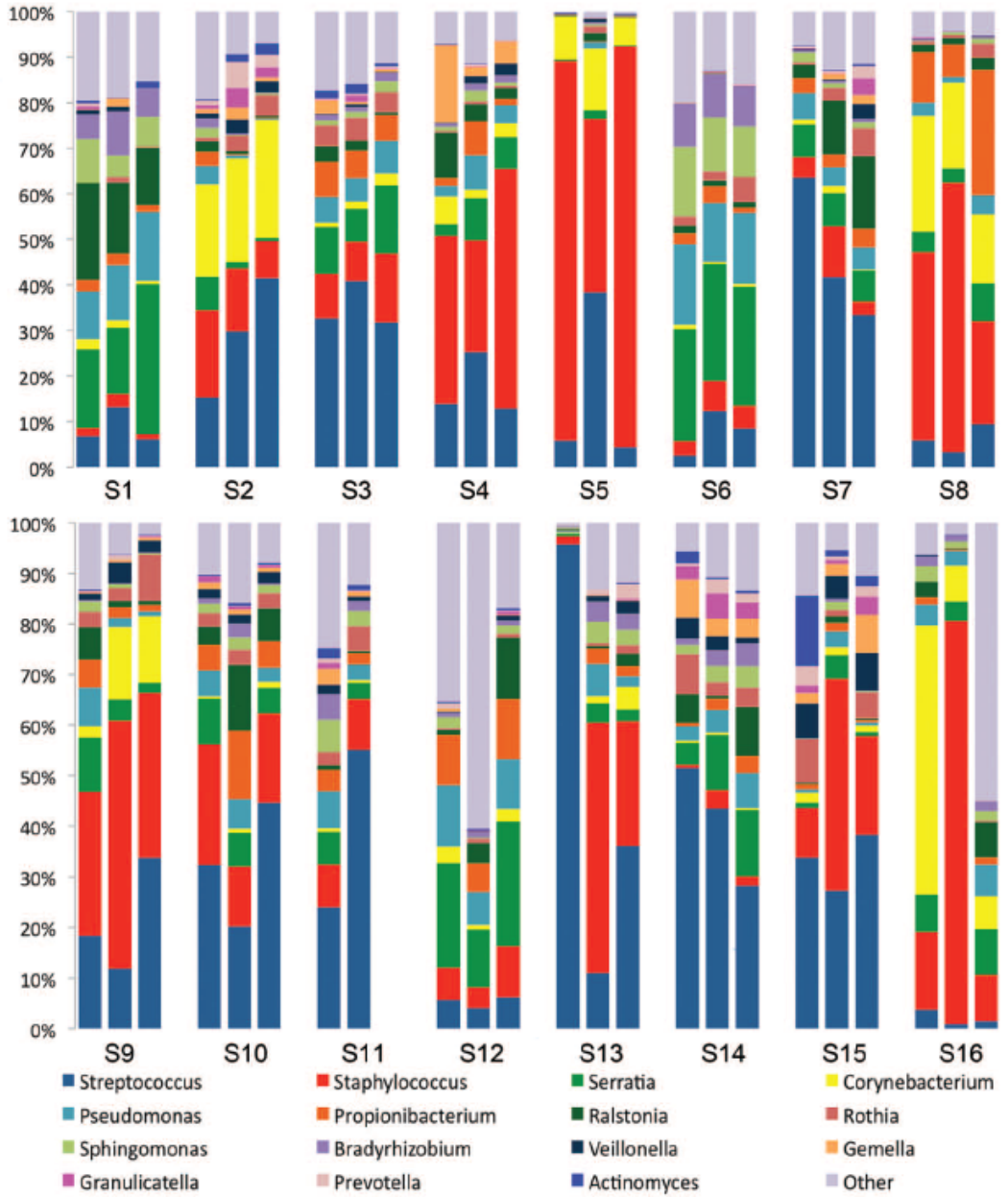
Relative HMO Composition (%)



(Lars Bode, Bianca Robertson, Azad et al. Unpublished)

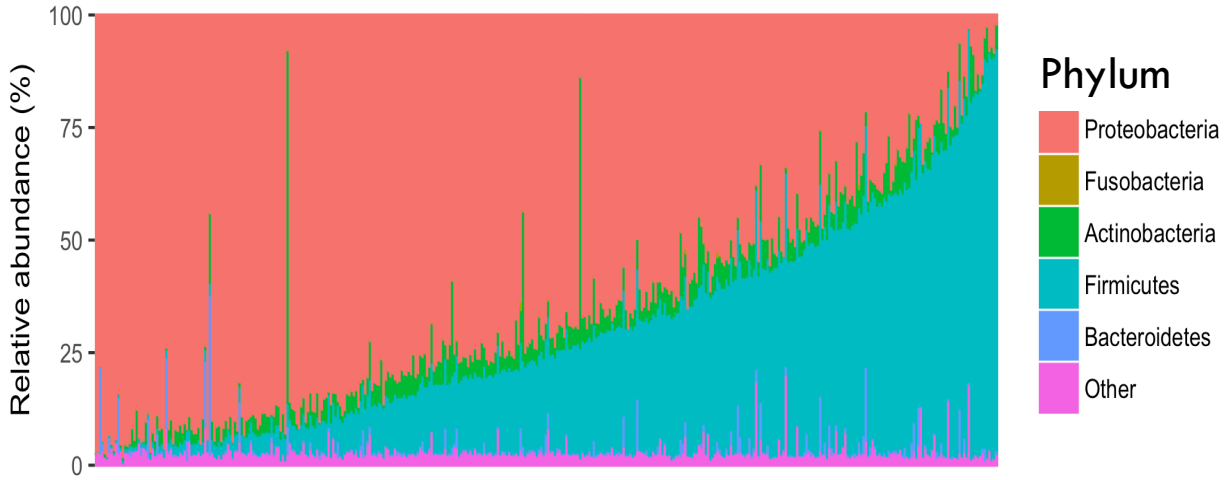
Milk Microbiota

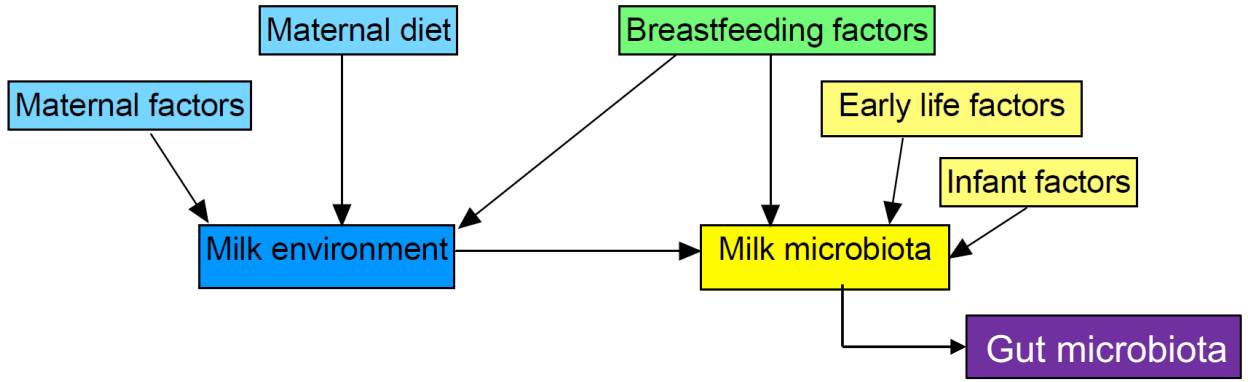
- Human milk is not sterile!
  - Breastfed infants consume 10^5–10^7 bacteria daily.
- Source of gut microbiota
- A few small studies (N < 30):
  - Variation by birth mode, obesity, time postpartum, gestational age, genetics, country… ???
  - **None** examined infant health

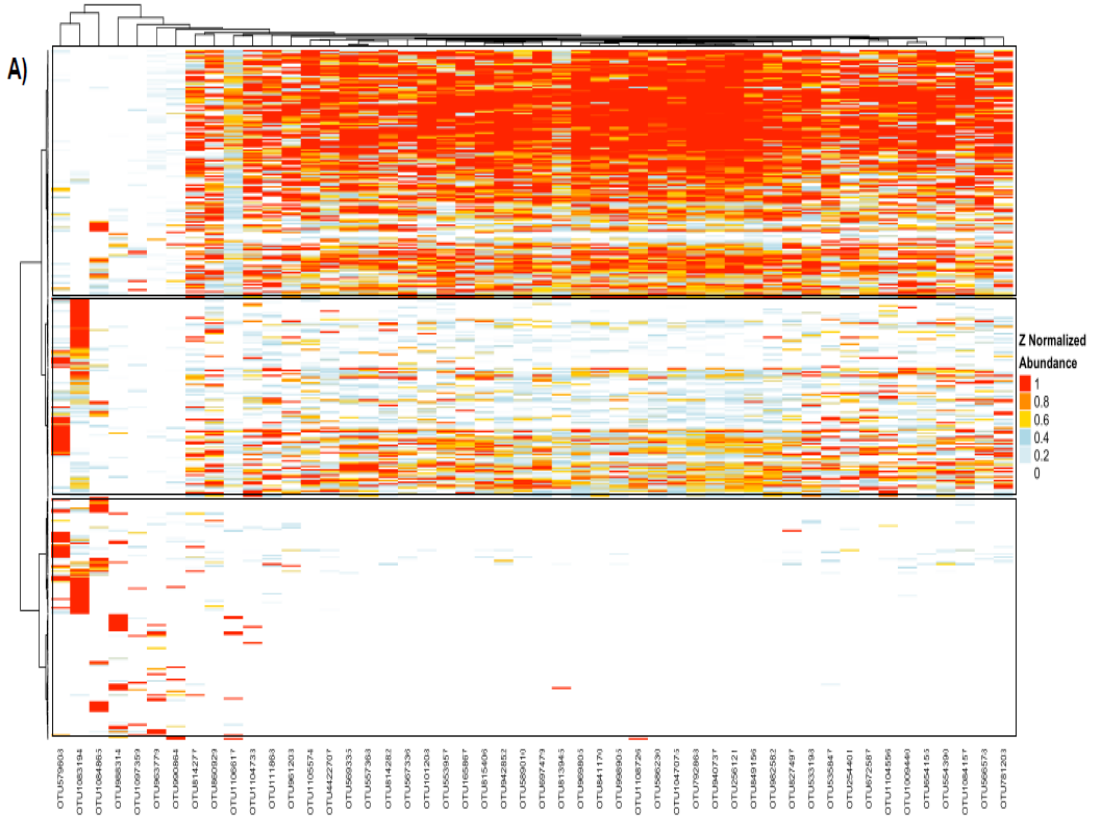


(Hunt et al. PLOS One 2011)

Milk Microbiota in the CHILD Cohort







(Shirin Moossavi, Ehsan Khafipour, Azad et al. Unpublished)

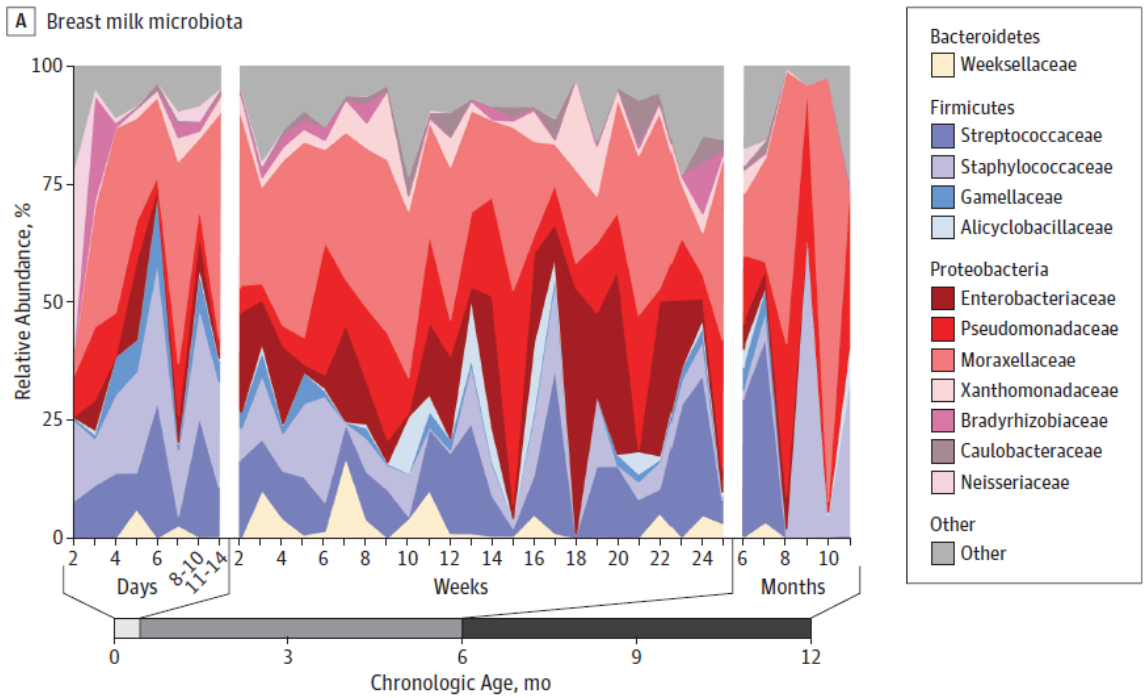
JAMA Pediatrics | Original Investigation

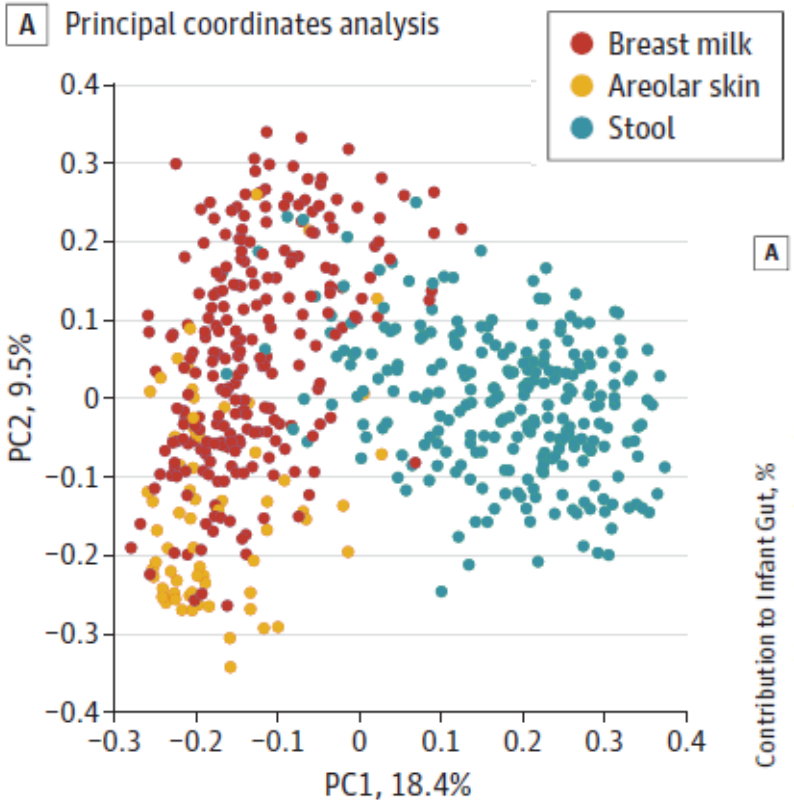
Association Between Breast Milk Bacterial Communities and Establishment and Development of the Infant Gut Microbiome

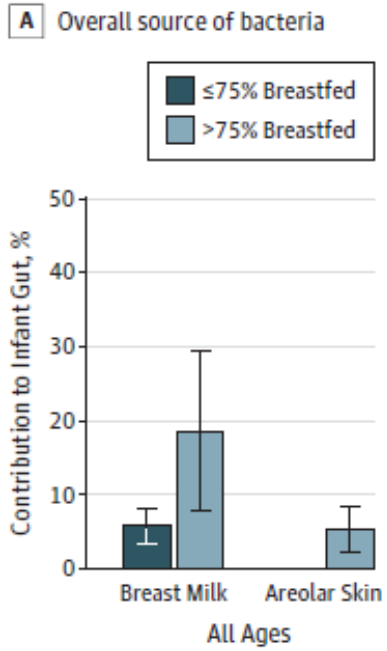
Pia S. Pannaraj, MD, MPH; Fan Li, PhD; Chiara Cerini, MD; Jeffrey M. Bender, MD; Shangxin Yang, PhD; Adrienne Rollie, MS; Helty Adisetiyo, PhD; Sara Zabih, MS; Pamela J. Lincez, PhD; Kyle Bittinger, PhD; Aubrey Bailey, MS; Frederic D. Bushman, PhD; John W. Sleasman, MD; Grace M. Aldrovandi, MD

JAMA Pediatr. doi:10.1001/jamapediatrics.2017.0378
Published online May 8, 2017.

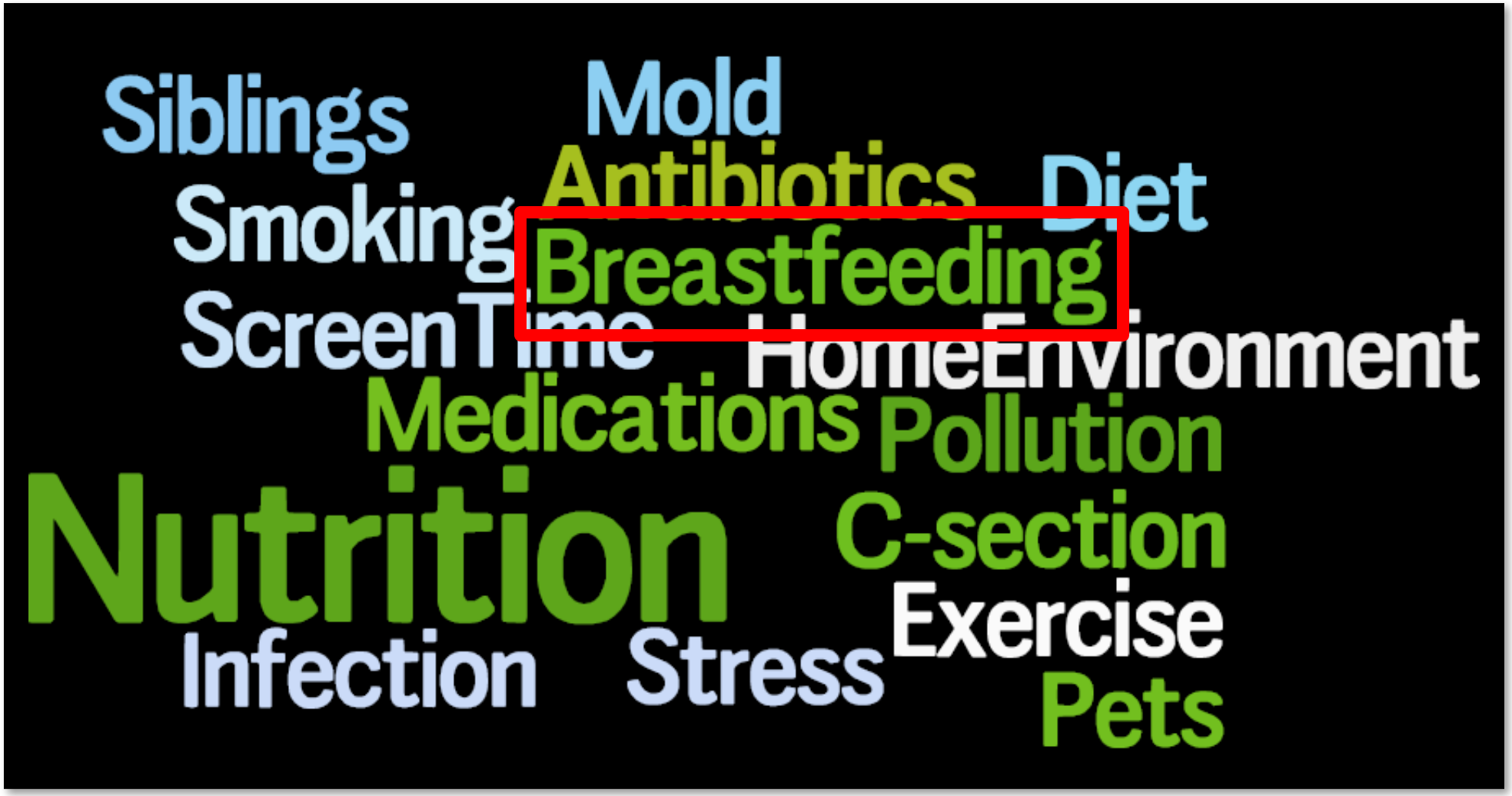
N=107 mother-infant pairs (USA)





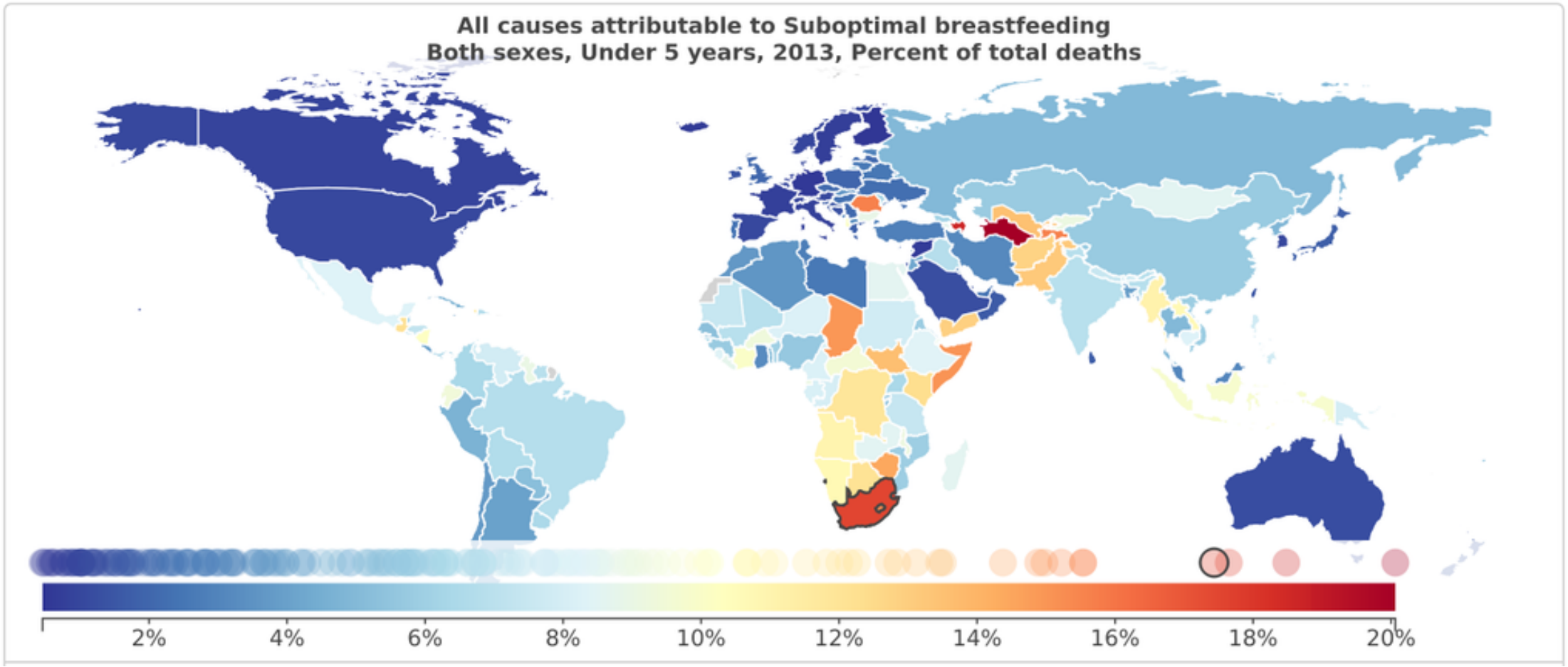


What early life factors contribute to health disparities?



Suboptimal breastfeeding & child mortality

IHME
Institute for Health Metrics and Evaluation



http://vizhub.healthdata.org/gbd-compare

Breastfeeding Inequities

Less Breastfeeding:

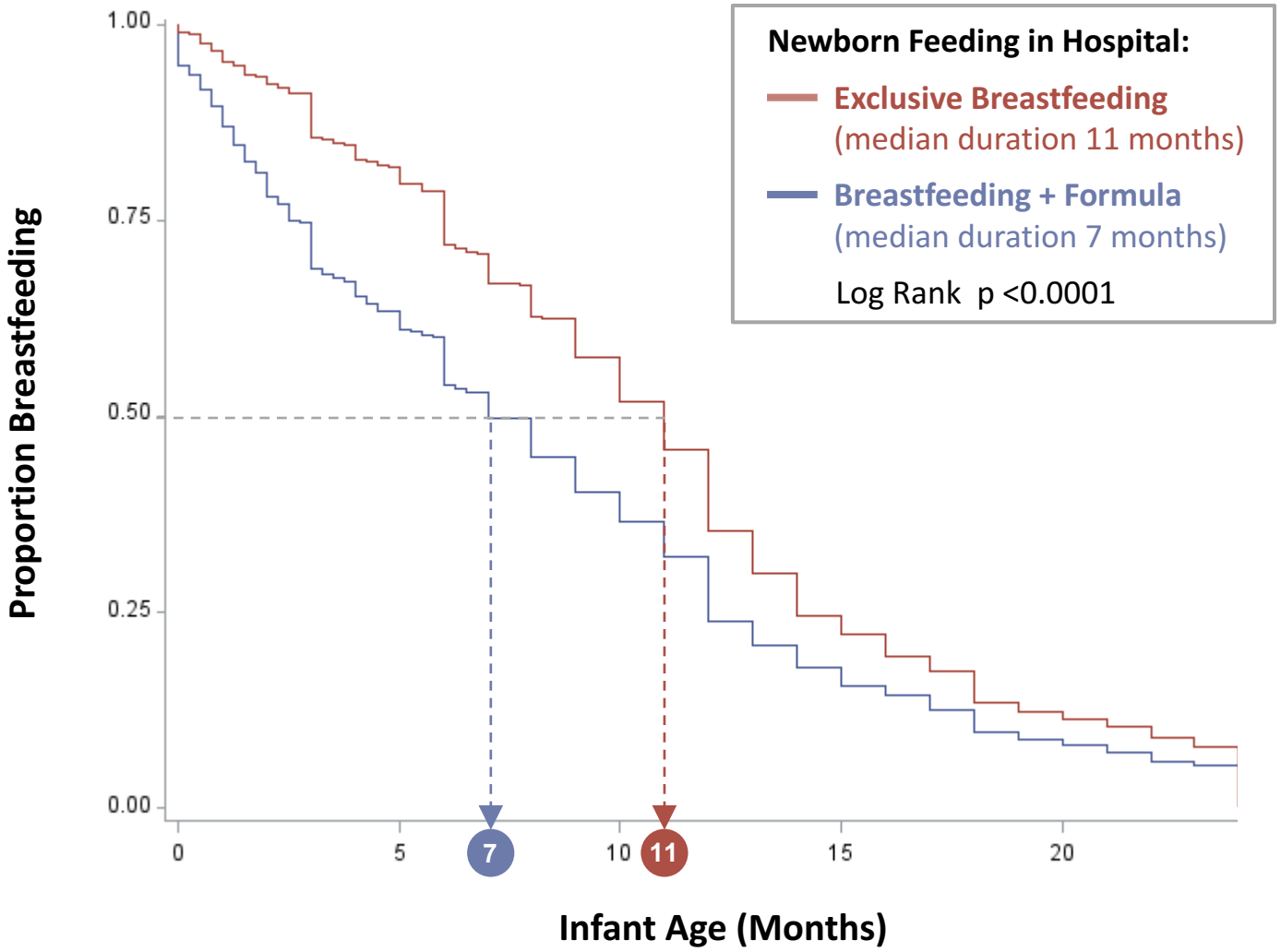
- Lower education
- Maternal obesity
- Maternal smoking
- First Nations Ethnicity
- Younger maternal age

More Breastfeeding:

- Vancouver

	N	Exclusive BF at 6 months %	p	Any BF at 12 months %	p
Site					
Edmonton	781	15.2	***	35.9	***
Toronto	777	16.6		37.4	
Vancouver	740	25.3		60.9	
Winnipeg	998	16.3		39.4	
Maternal age (years)					
<30	991	12.8	***	32.6	***
30 to 35	1372	18.4		45.9	
35+	910	23.6		49.8	
Pre-pregnancy BMI (kg/m^2)					
Normal: <25	1863	19.6	**	49.8	***
Overweight: ≥25 - 30	633	18.9		43.9	
Obese: ≥30	440	12.6		27.0	
Ethnicity					
Asian	508	19.5		49.0	*
Caucasian	2359	18.7		42.8	
First Nations	143	14.0		35.3	
Other	225	12.0		39.6	
Education					
≤ High school	280	8.7	***	24.5	***
Some post-secondary	466	15.1		36.8	
Post-secondary	1805	18.8		43.9	
Post-graduate	602	23.6		55.8	

Supporting Breastfeeding



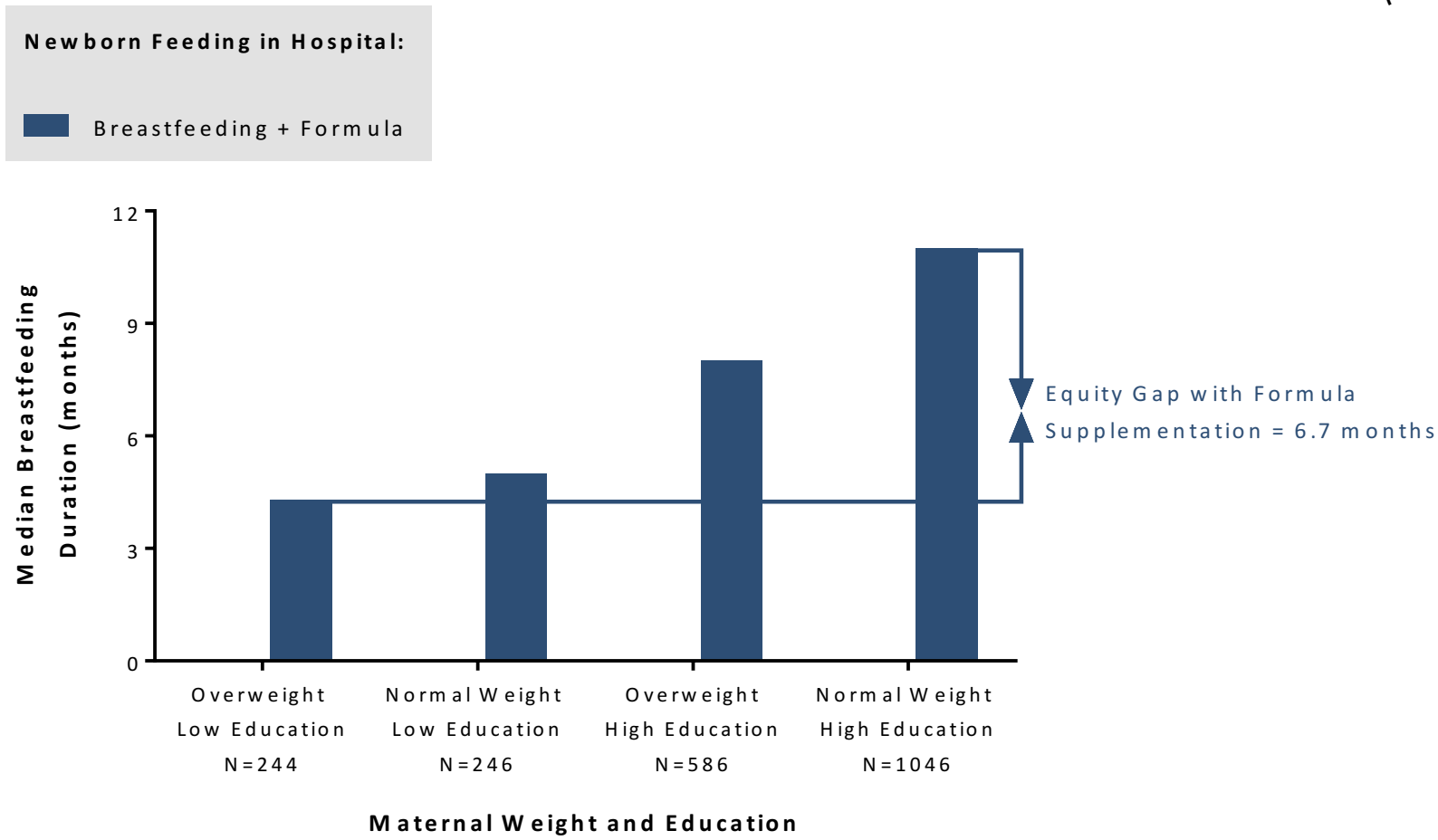
Compared to newborns who received formula supplementation, those who were exclusively breastfed in hospital breastfed **4 months longer** and had a **21% reduced risk** of breastfeeding cessation over time. (HR 0.79; 95%CI: 0.72-0.88)

Accredited as Baby Friendly by unicef

(Vehling et al. Submitted)

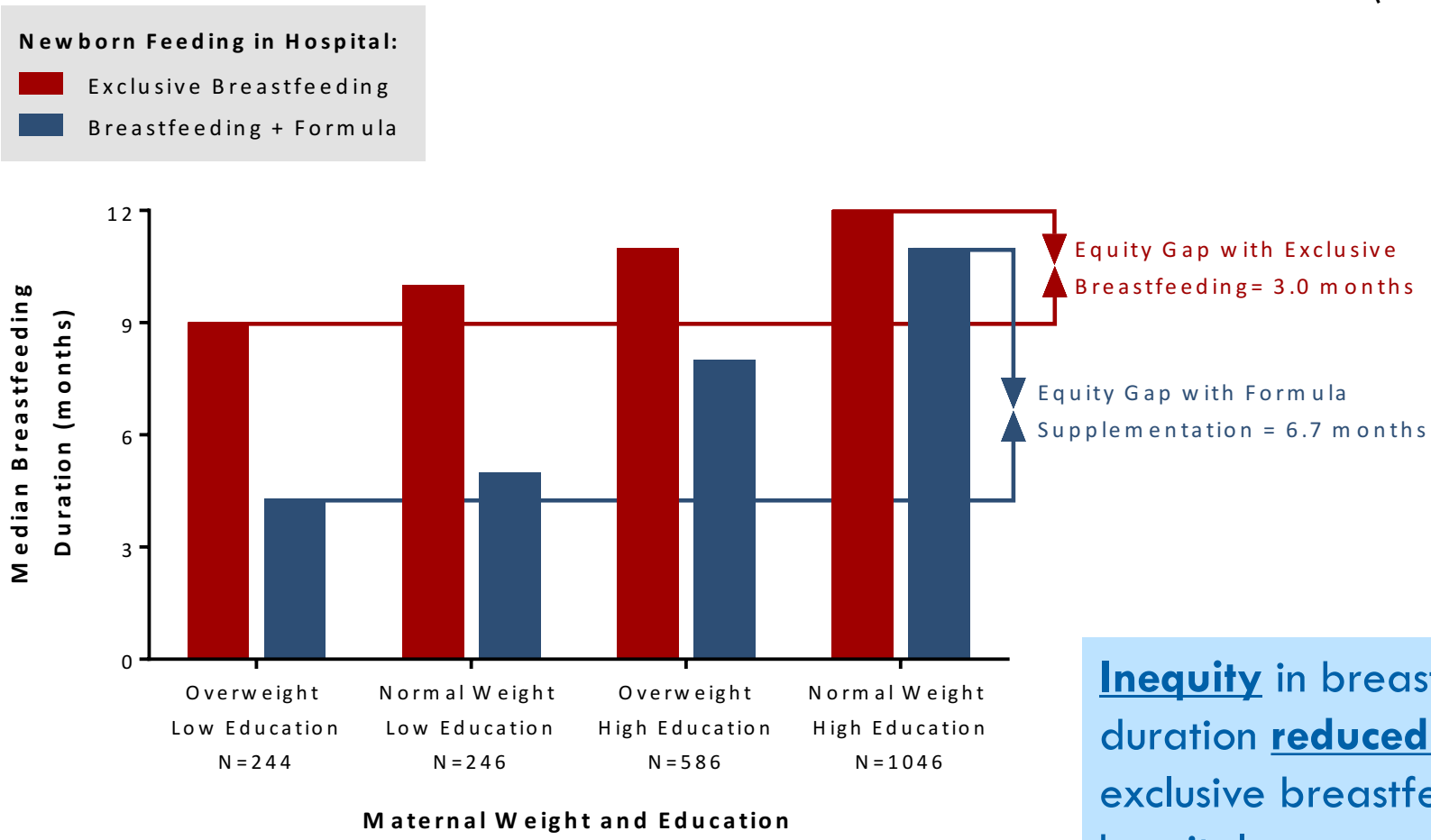
Newborn Feeding & Breastfeeding (in)Equity

(Vehling, Azad et al. Submitted)



Newborn Feeding & Breastfeeding (in)Equity

(Vehling, Azad et al. Submitted)



Inequity in breastfeeding duration **reduced by 55%** with exclusive breastfeeding in hospital.

Summary: DOHaD / Gut Microbiota / Disparities

- Early **risk** factors:
  - Cesarean section
  - Antibiotics
  - Tobacco smoke
  - Maternal stress
- Early **protective** factors:
  - (Direct) Breastfeeding
  - Pets

Unanswered Questions…

- **What is a 'healthy microbiome'?**
- What are the **long-term** health effects of microbiota "dysbiosis" resulting from early life exposures?
- **HOW** do microbiota influence disease risk?
- How can we **prevent** gut microbiota dysbiosis?
  - Vaginal delivery, **breastfeed**, avoid unnecessary antibiotics…
- How can we **repair** gut microbiota dysbiosis?
  - "Vaginal Seeding" after CS? **Breastfeed**? Pre/probiotics? Fecal Transplants?
- How can we target gut microbiota (in early life) to **reduce health disparities**?

Developmental Origins of CHILD HEALTH & Disease

Meghan Azad, PhD

Breastfeeding

Mechanisms

- Gut Microbiota
- Epigenetics
- Metabolism
- Lung Function
- Immunity

Allergies, Asthma, Obesity, Diabetes, Cognitive Development…

Interventions

Milk Composition

- Microbiota
- Oligosaccharides
- Immune Factors
- Fatty Acids
- Hormones
- Vitamins

Maternal Factors

Modifiable: Obesity, Nutrition, Self-Efficacy, Smoking, Birth Mode, **Pro/Antibiotics… Fixed:** Age, Ethnicity, Genetics, **Asthma…**

the Children's Hospital Research Institute of Manitoba

DEVOTION
Developmental Origins of Chronic Diseases in Children Network

University of Manitoba

Acknowledgements

Azad Lab
Faisal Atakora, Kozeta Miliku, Michelle La,
Lorena Vehling, Deborah Chan, Hasantha Sinnock,
Annika Klopp

CHILD Study
Manitoba: Allan Becker & Team
National: Malcolm Sears (McMaster) & Team
SyMBIOTA: Anita Kozyrskyj (U. Alberta), James Scott
(U. Toronto) & Team

Collaborators
Ehsan Khafipour, Shirin Moosavi (U. Manitoba)
Lars Bode (U. California San Diego)

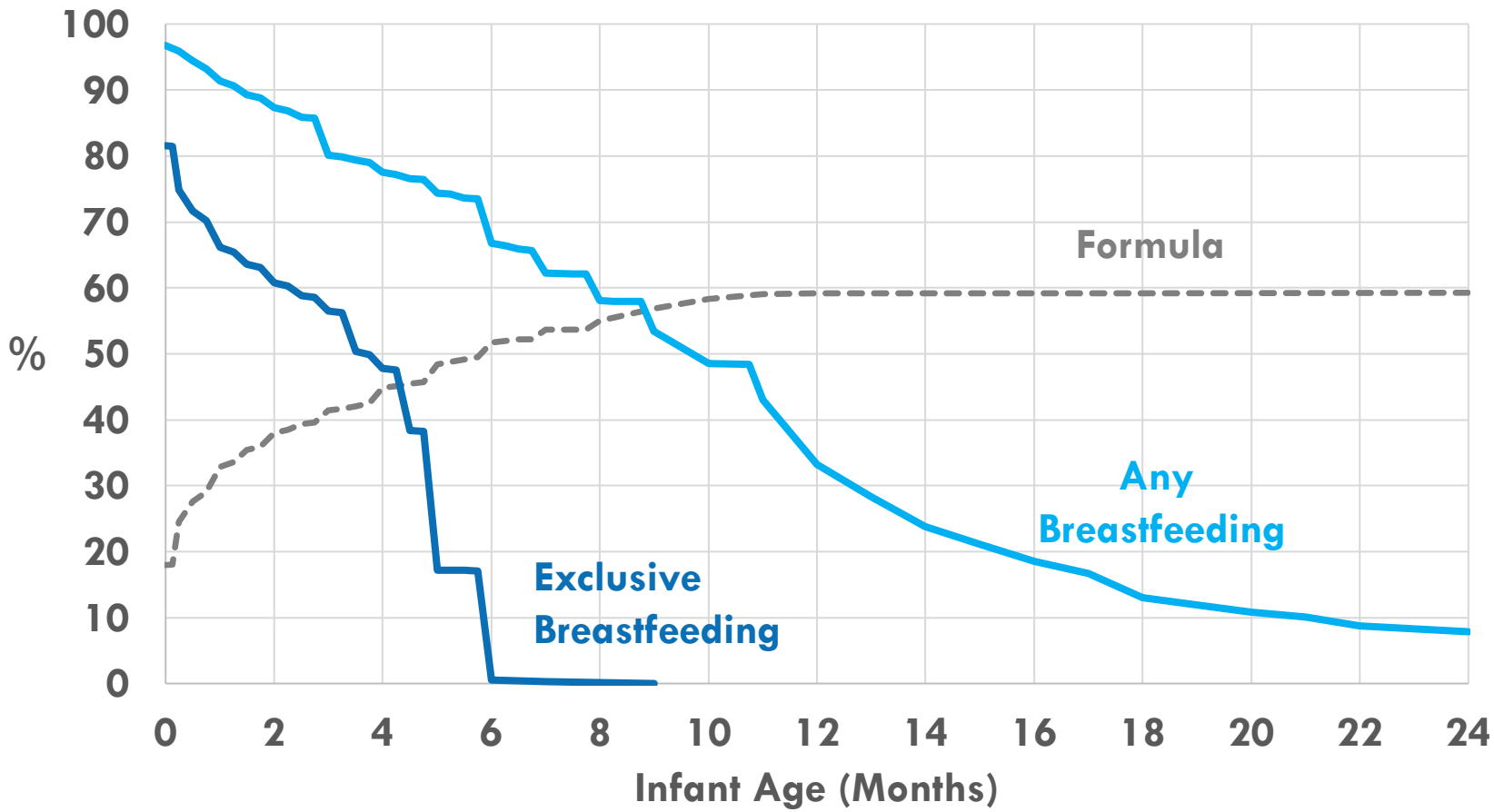
CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada
AllerGen Allergy, Genes and Environment Network
DEVOTION Manitoba Developmental Origins of Chronic
Research Manitoba
Healthy Child Manitoba Putting children and families first
Manitoba
the Children's Hospital Foundation of Manitoba
CRRN RCRR Canadian Respiratory Research Network / Réseau canadien de recherche respiratoire

"If breastfeeding did not already exist, someone who invented it today would deserve a ***dual Nobel Prize*** *in medicine and economics.*

Breastfeeding is a child's first inoculation against death, disease, and poverty, but also their most enduring investment in physical, cognitive, and social capacity."

Keith Hudson
VP Human Development
World Bank Group

Breastfeeding in the CHILD Study



World Health Organization

Recommends:

Exclusive BF to **6 mos**

Continued BF to **2 yrs+**

N=3139 (Formula), 3159 (Any BF), 3057 (Exclusive BF)

(Vehling, Azad et al. Unpublished)