Nutrition and Intestinal Microbiota in Infancy

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Ingestion of Bacteria Proposed as Beneficial

Elie Metchnikoff
(1845-1916)

- Suggested that ingested bacteria could have positive influence on normal microbial flora in intestinal tract
- Hypothesized that Lactobacilli were important for human health and longevity
- Promoted yogurt and fermented foods as healthy

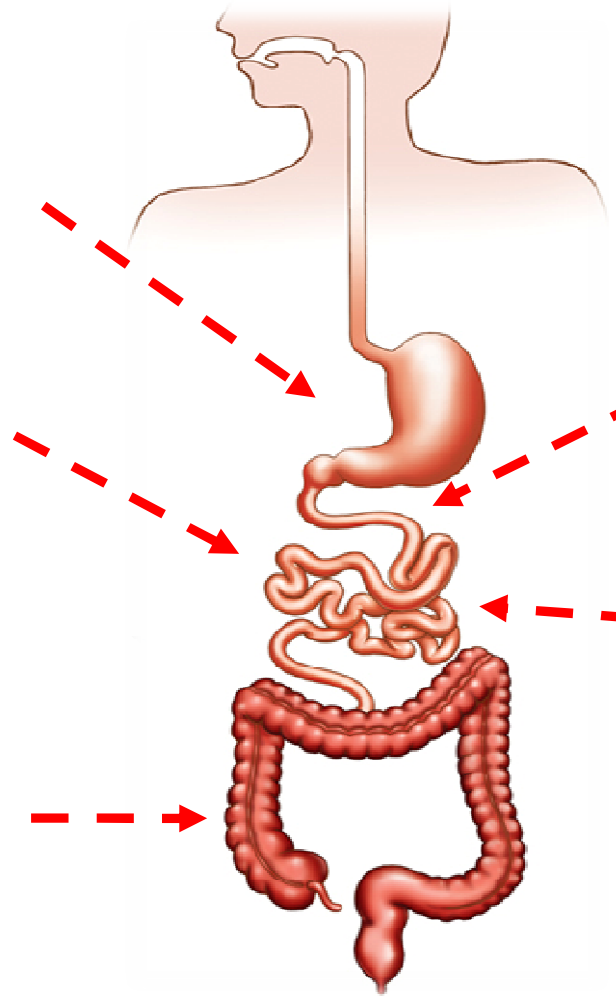
Bacteria in the GI Tract: Complex Ecosystem

Resident and Ingested

Stomach
10^4 CFU/g
Candida albicans
Helicobacter pylori
Lactobacillus
Streptococcus

Jejunum
10^5-10^7 CFU/g
Bacteroides
Candida albicans
Lactobacillus
Streptococcus

Colon
10^{10}-10^{11} CFU/g
Bacteroides
Bacillus
Bifidobacterium
Clostridium
Enterococcus
Eubacterium
Fusobacterium
Peptostreptococcus
Ruminococcus
Streptococcus



500 – 1000 species

Duodenum
10^3-10^4 CFU/g
Bacteroides
Candida albicans
Lactobacillus
Streptococcus

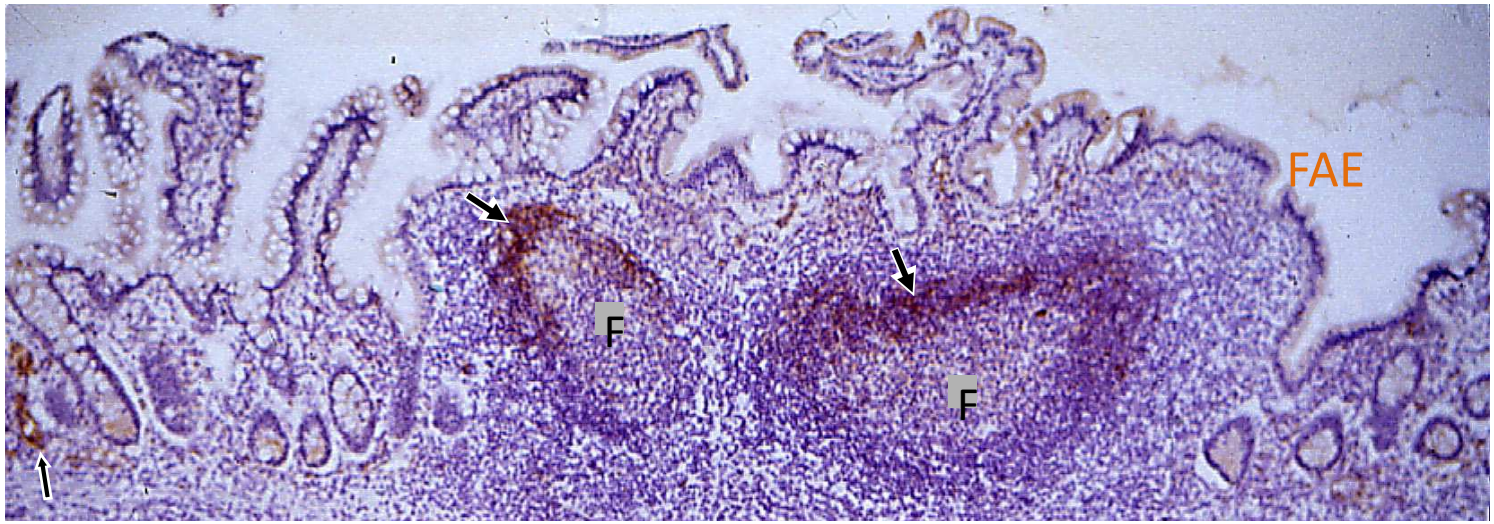
Ileum
10^7-10^8 CFU/g
Bacteroides
Clostridium
Enterobacteriaceae
Enterococcus
Lactobacillus
Veillonella

Homo Bacteriensis?

Intestinal Microbiota

- One of the most densely populated microbial ecosystems on Earth
- 100 trillion cells (10 x # of host cells)
- 3,000,000 encoded genes: complement host's metabolic pathways
- 4 dominant phyla; ~1000 species; ~10, 000 strains ("the microbiome")
- Most (approximately 80%) have not yet been cultivated

GUT ASSOCIATED LYNPHOID TISSUE

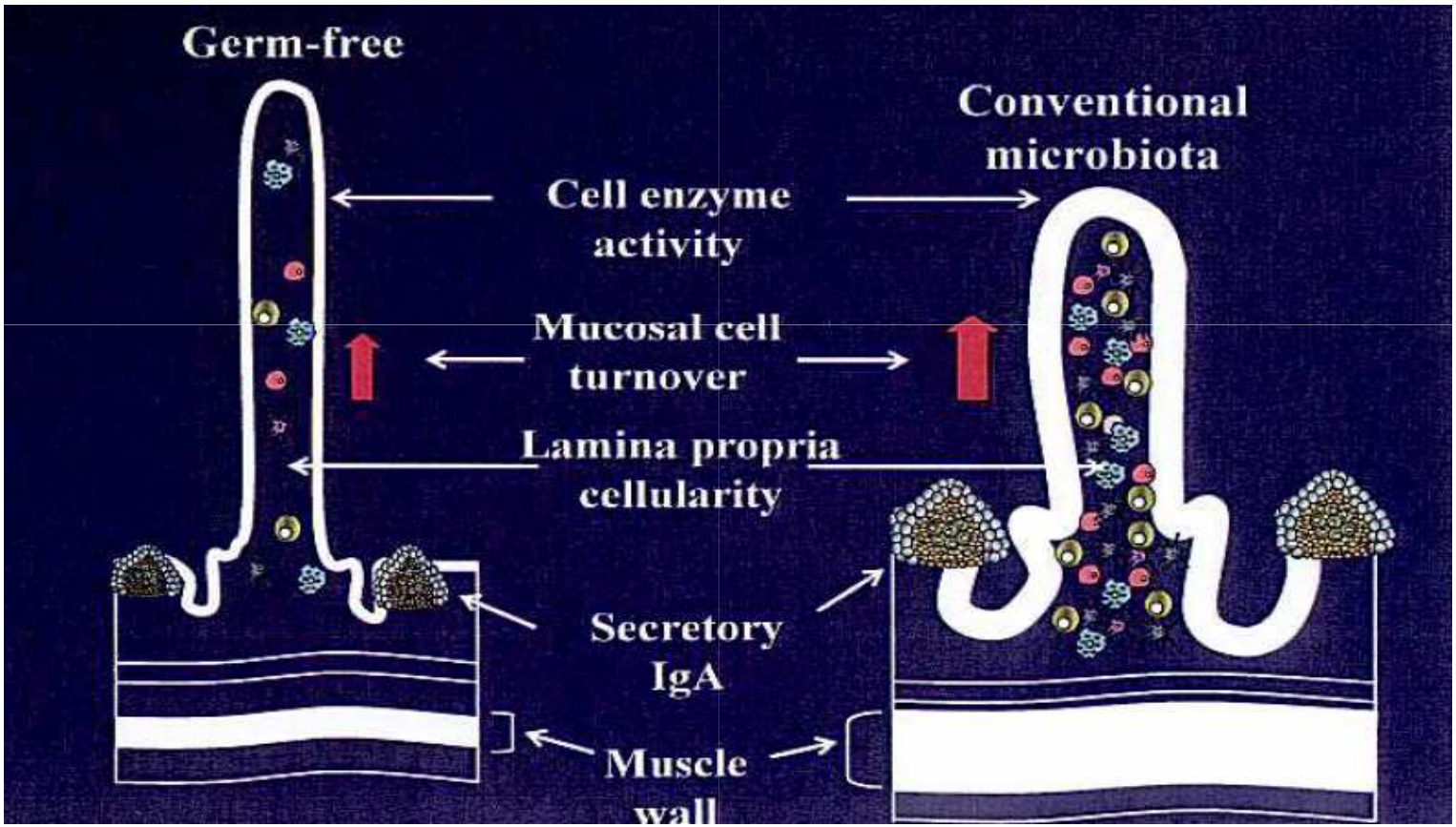


Gut-Associated Lymphoid Tissue structures are strategically situated in proximity to the greatest concentration of microbiota

- Peyer's patches: distal ileum (nos. 100-250)
- Isolated lymphoid follicles (ILFs): large bowel (nos. ~ 30 000)
- **70% of immunologically active cells in the body**

Brandtzaeg, Immunological Investigations 2010

Germ-free vs. Colonized Gut
Bacteria Stimulate Normal Immune response



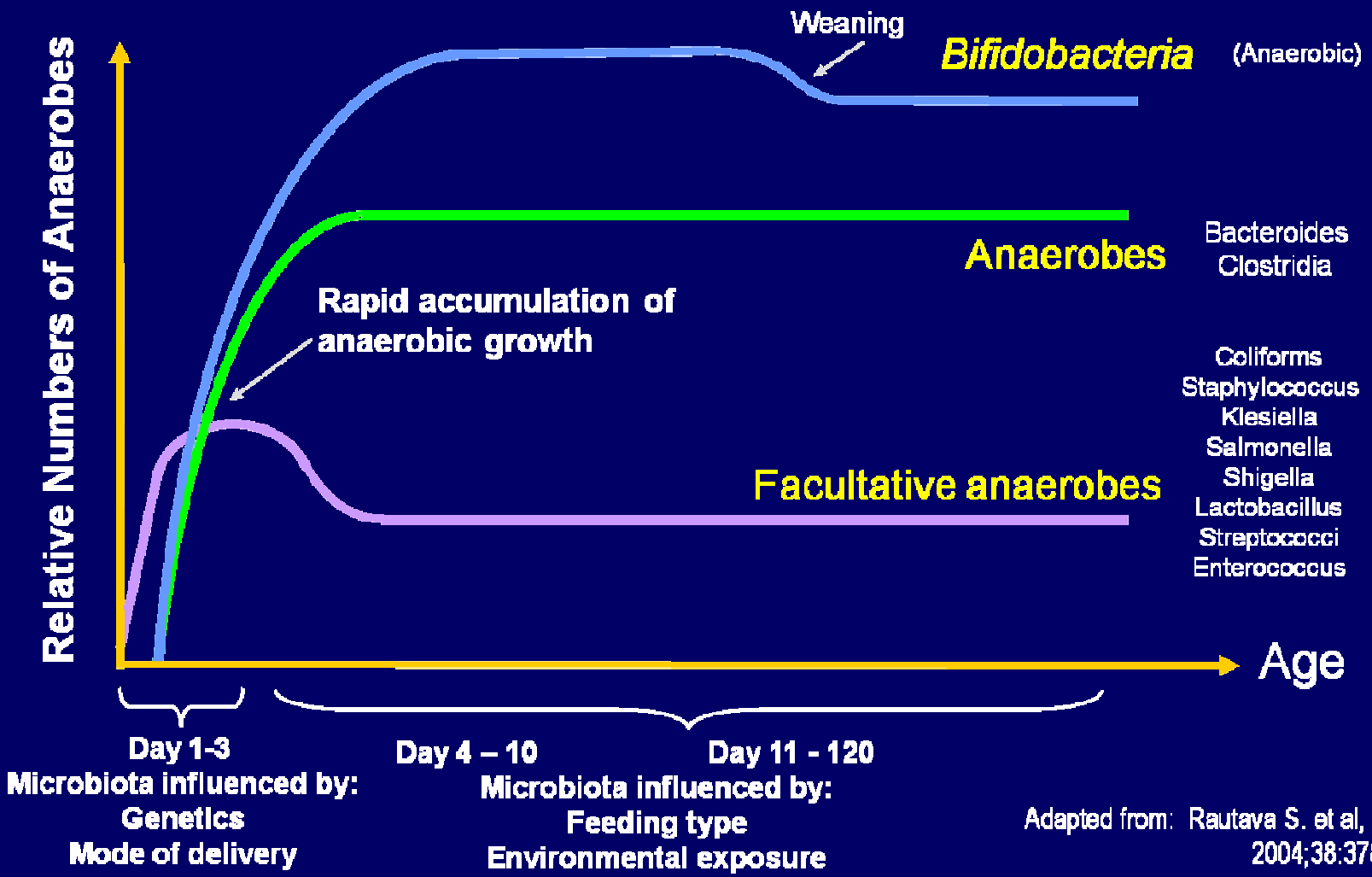
A healthy microbiota is critical to train the immune system to protect the host and decrease the chances for immune over expression (immune related conditions).

Function GI microbiota

Immune development & modulation	• IgA production • Control of local and general inflammation • Tightenning of intercellular juntions • Induction of tolerance to foods
Pathogen protection	• Pathogen displacement / Nutrient competition • Production of mucin & antimicrobial factors • Activation of local immune response • Contribution to the intestinal barrier function
Digestive and metabolic functions	• Vitamin production • Fermentation of non-digestible carbohydrates • Dietary carcinogens metabolism
Neuronlogic development and function	• Modulation of brain gut axis during neuronal develoment • Motor control and anxiety behavious

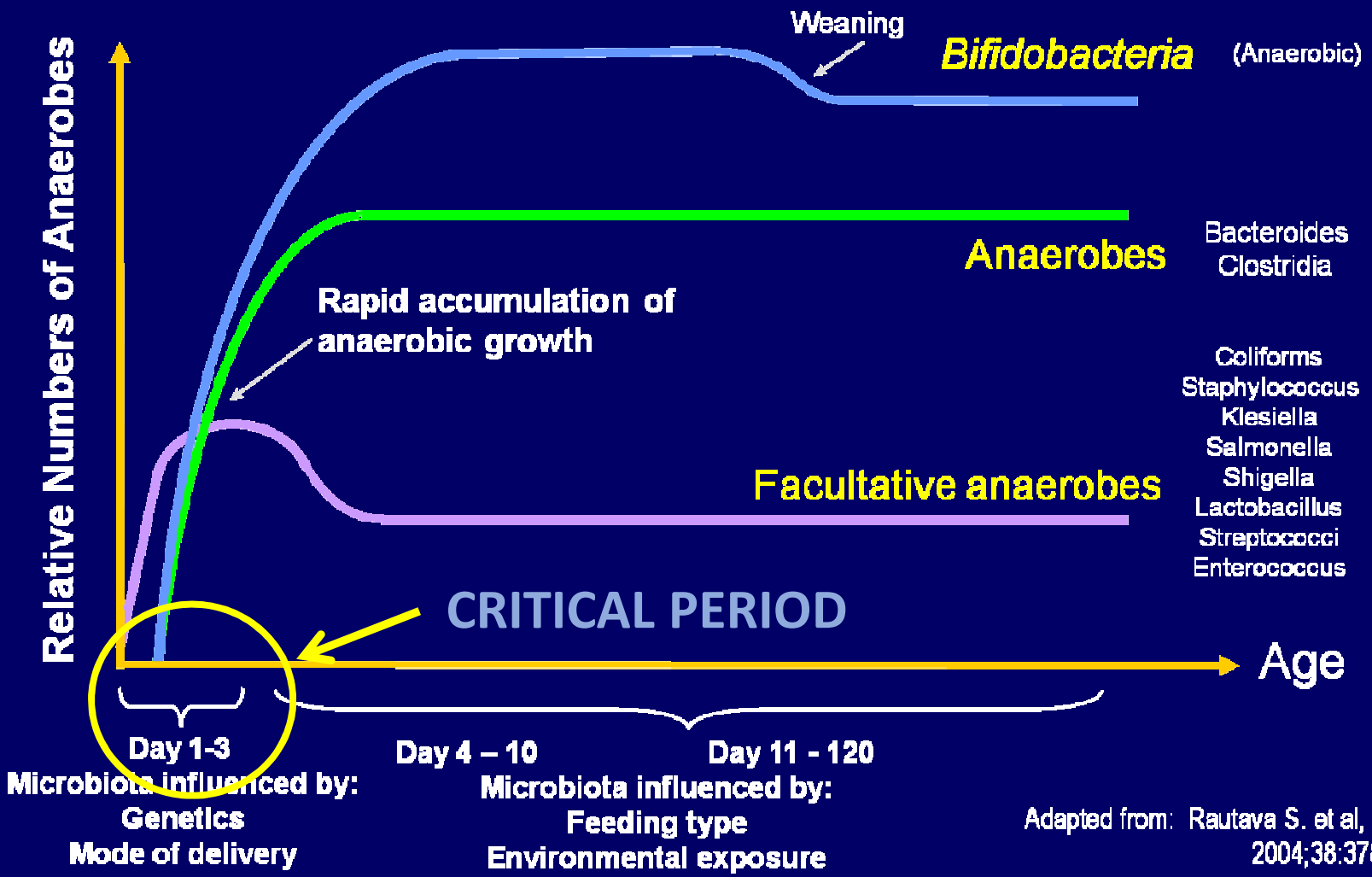
Adapted (JMS) from Buccigrossi et al. Curr Opin Gastroenterol 2013;29:31-8.

Development of Intestinal Microbiota



Adapted from: Rautava S. et al, JPGN 2004;38:378-388.

Development of Intestinal Microbiota



Adapted from: Rautava S. et al, JPGN 2004;38:378-388.

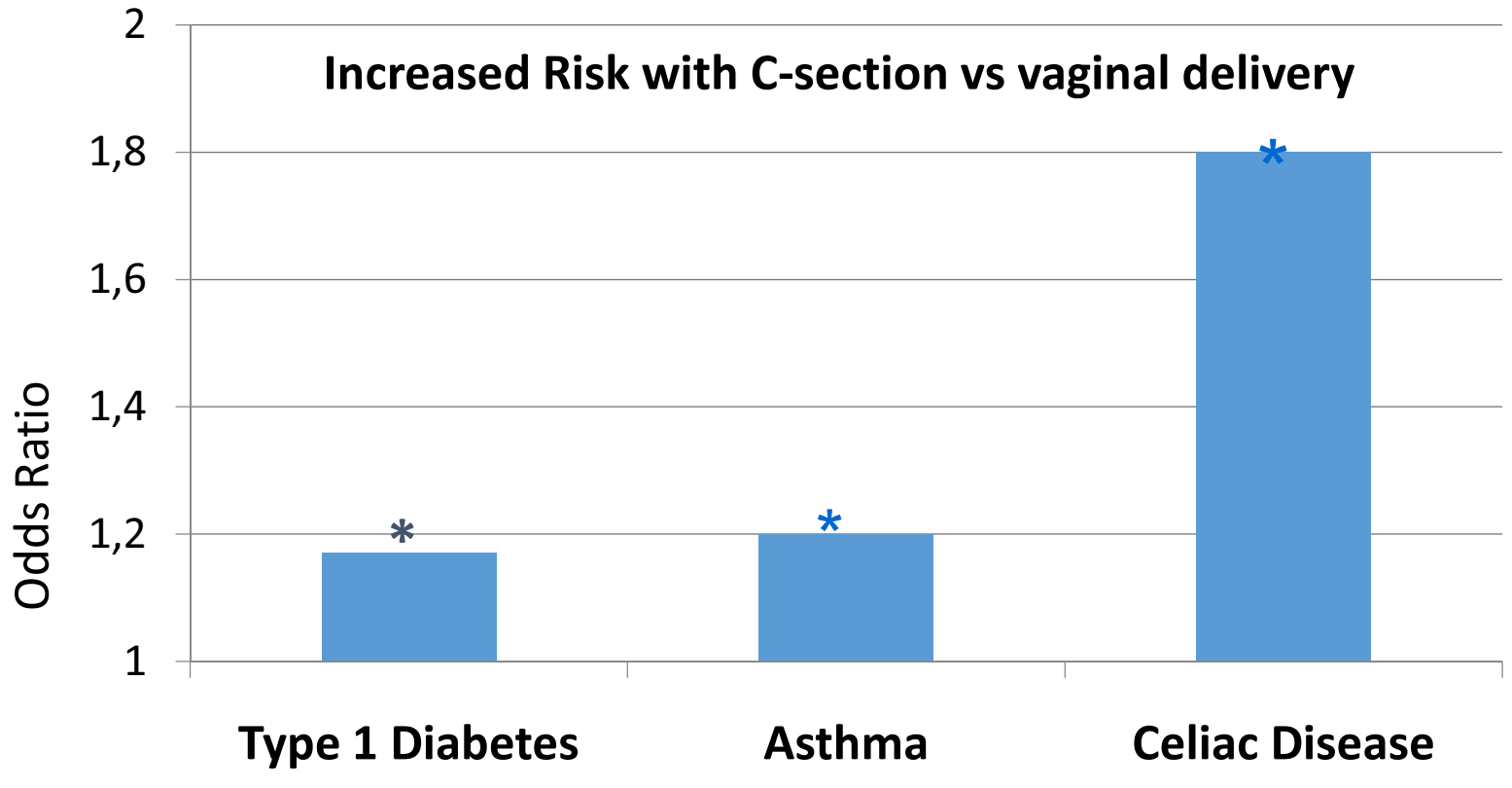
Sources of bacteria for the newborn:

- The vaginal canal and normal delivery
- Breastmilk and breastfeeding

Vaginal delivery is not a sterile procedure

Are there immunologic consequences to a sterile birth (C-section)?

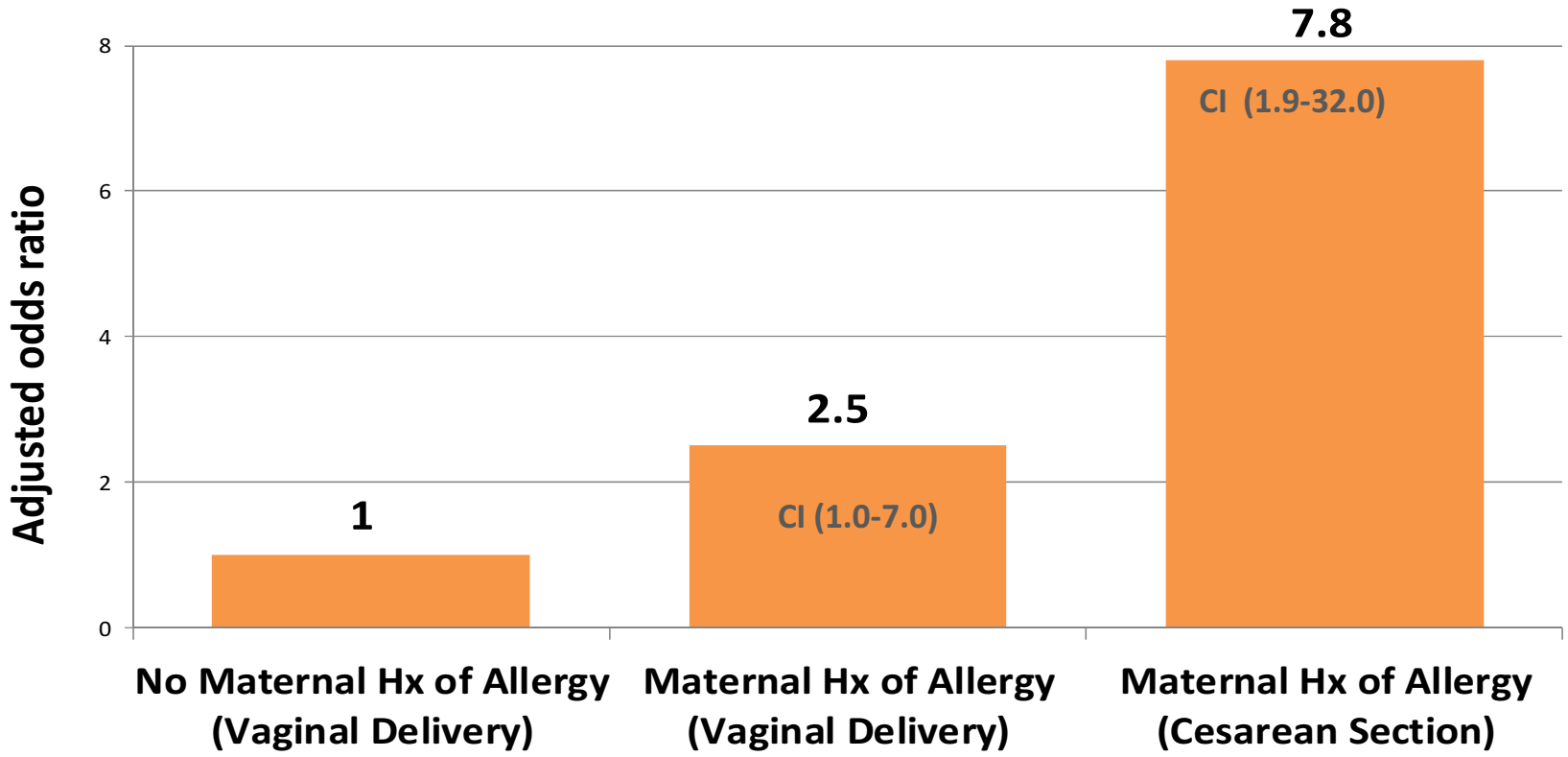
Cesarean Delivery Linked to Increases in **Chronic Disease**



* p< .05

D'Angeli MA, et al. Arch Pediatr Adolesc Med. 2010 Aug;164(8):732-8.
Davidson R, et al. BMC Pulm Med. 2010 Mar 16;10:14.
Decker E, et al. Pediatrics. 2010 Jun;125(6):e1433-40.

Cesarean Delivery and Relative Risk of Childhood **Food Allergy**

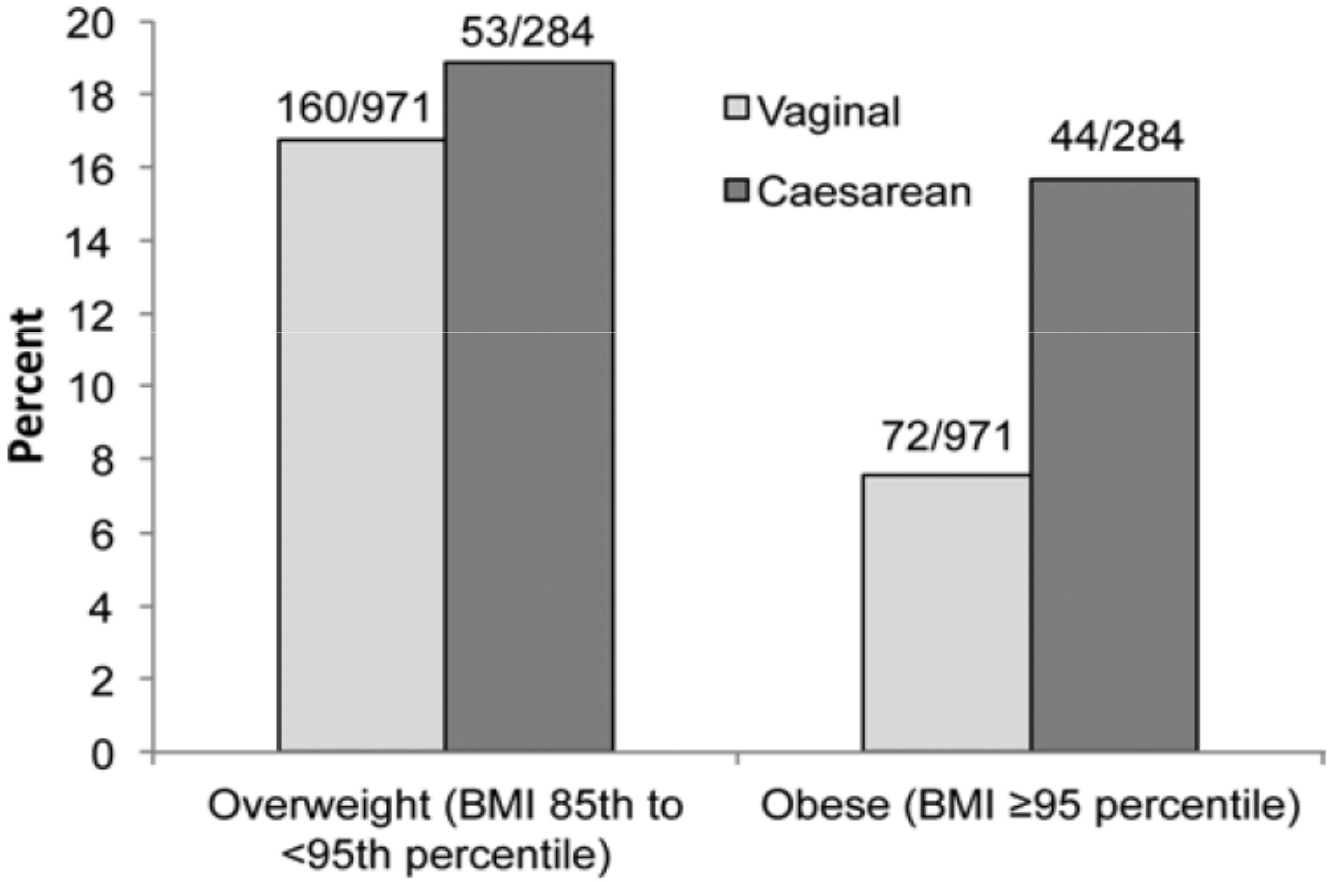


Pistiner M et al. J Allergy Clin Immunol 2008; 122(2):274-279
Table adapted from: Eggesbo M et al. J Allergy Clin Immunol 2003;112:420-426

***p<0.01; adjusted for covariates**

Food Allergy to egg confirmed by testing at age 1 – 2 y

Cesarean Section Linked to Increases in Chronic Disease: Childhood Obesity



Huh SY et al, Arch Dis Child published online May 2012

Microbiota in C-section infants

- C-section infants
- Minimal or no vaginal microbes
- Low Lactobacillus, Prevotella, Sneathia spp (vaginal microbes)
- Higher skin bacteria
  (e.g., Staphylococcus, Corynebacterium, Propionibacterium spp.)
- Higher levels of C. difficile
- Lower and delayed appearance of Bacteroides and Bifidobacterium spp.

Breastfeeding is not a sterile procedure

Are there consequences to exclusive formula feeding?

Breastfeeding is Consistently Associated with Infant Health Outcomes

	Breastfeeding is associated with:
Infectious Disease	↓ Acute otitis media[1]
	↓ Non-specific gastroenteritis[1]
	↓ Severe lower respiratory tract infections[1]
Immunologic Disease	↓ Atopic dermatitis[1]
	↓ Asthma (young children)[1]
	↓ Type 1 diabetes[1]
	↓ IBD (Crohn disease, U. Colitis)
	↓ Childhood leukemia[1]
Metabolic Disease	↓ Obesity and Type 2 diabetes[1]

I. *Evid Rep Technol Assess (Full Rep)*. 2007 Apr;(153):1-186; 2. Anderson JW, et al. *Am J Clin Nutr*. 1999;70:525-35; 3. Kramer MS. *Early Hum Dev*. 2010;86:729-32; 4. Kramer MS, et al. *Arch Gen Psychiatry*. 578-84; 5. Daniels MC, Adair LS. *J Nutr*. 2005;135:2589-95. 6. Knip M, et al. Am J Clin Nutr. 2010;91(5):1506S-1513S. 7. Klement E, et al. Am J Clin Nutr. 2004;80(5):1342-52.

Nutrition in early life sets the course for long term health of individuals and all society

- Breastfeeding is the best start to nutrition and health – for life
- Exclusive breastfeeding in the first months of life provides unique benefits to infants and others

Breastmilk remains the model that inspires the way infants should be fed

COMPOSITION of HUMAN MILK (not exhaustive …)

HUMAN MILK

OHYDRATES
se
saccharides (>200)

EINS
(including amino acids)
talbumin
ns
n Albumin
rotein Nitrogen
ne
nine
cid
otides
H
GA

TH FACTORS
IL-2
L-6
L-10
F, M-CSF
, VEGF
α, HGF-β
TNF-α
, TGF β1
β2

PEPTIDES
- Whey peptides
- Casein peptides
- β-Defensin 1
- β-Endorphins
- Gastrin
- Motilin
- Neurotensin
- Somatostatin

HORMONES
- Insulin, Leptin
- Adiponectin
- Cortisol, T3, T4
- TSH, TRH, Prolactin
- Oxytocin, Ghrelin

ENZYMES
- BSSL
- Amylase
- Catalase
- Histaminase
- Phosphatase
- Lysozyme
- Xanthine Oxidase
- Antiproteases

IMMUNE FACTORS
- sIgA
- IgA2
- IgG
- IgD
- IgM
- IgE

LIPIDS
- Triaclyglycerols (TAG)
- Diacylglcerols (DAG)
- Monoacylglycerols (MAG)
- Fatty acids (FA; esterified & free)
  - SFA (16:0)
  - MUFA (18:1)
  - PUFA n-3 (ALA)
  - PUFA n-6 (LA, DHA)
  - MCFA (10:0, 12:0)
  - LCFA (18:0, 20:0)

PHOSPHOLIPIDS
- Phosphatidylcholine
- Sphingomyelin
- Phospatidylethanolamine
- Phosphatidylserine
- Phosphatidyliniositol
- Lyso-phospholipids
- Plasmalogens

SPHINGOLIPIDS
- Gangliosides (GM1, GM3, GD3)
- Glycosphingolipids
- Ceramides
- Glucosylceramides
- Galactosylceramides

STEROLS
- Cholesterol
- Squalene
- Lanasterol
- Sitosterol
- Dimethylsterol

MINERALS
- Na (Sodium)
- Mg (Magnesium)
- P (Phosphorus)
- K (Potassium)
- Ca (Calcium)
- Fe (Iron)
- Mn (Manganese)
- Cu (Copper)
- Zn (Zinc)
- Se (Selenium)
- I (Iodine)

VITAMINS
- Vitamin A, Vitamin B6
- Vitamin B9, Vitamin B12
- Vitamin C, Vitamin D
- Vitamin E, Vitamin K
- Pantothenic Acid
- Folic Acid, Carotenoids
- Pantothenic acid
- Folic acid
- Niacin, Biotin
- Choline, Inositol

CELLS AND OTHERS
- Leukocytes
- Macrophages
- Lymphocytes
- Stem Cells
- mRNA
- microRNA

man milk is a 'linving fluid' that can be compositionally emulated but not duplicated

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ne
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H
A
TH FACTORS
IL-2
L-6
L-10
F, M-CSF
, VEGF
α, HGF-β
TNF-α
, TGF β1
2

PEPTIDES
- Whey peptides
- Casein peptides
- β-Defensin 1
- β-Endorphins
- Gastrin
- Motilin
- Neurotensin
- Somatostatin

HORMONES
- Insulin, Leptin
- Adiponectin
- Cortisol, T3, T4
- TSH, TRH, Prolactin
- Oxytocin, Ghrelin

ENZYMES
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- Catalase
- Histaminase
- Phosphatase
- Lysozyme
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- Lyso-phospholipids
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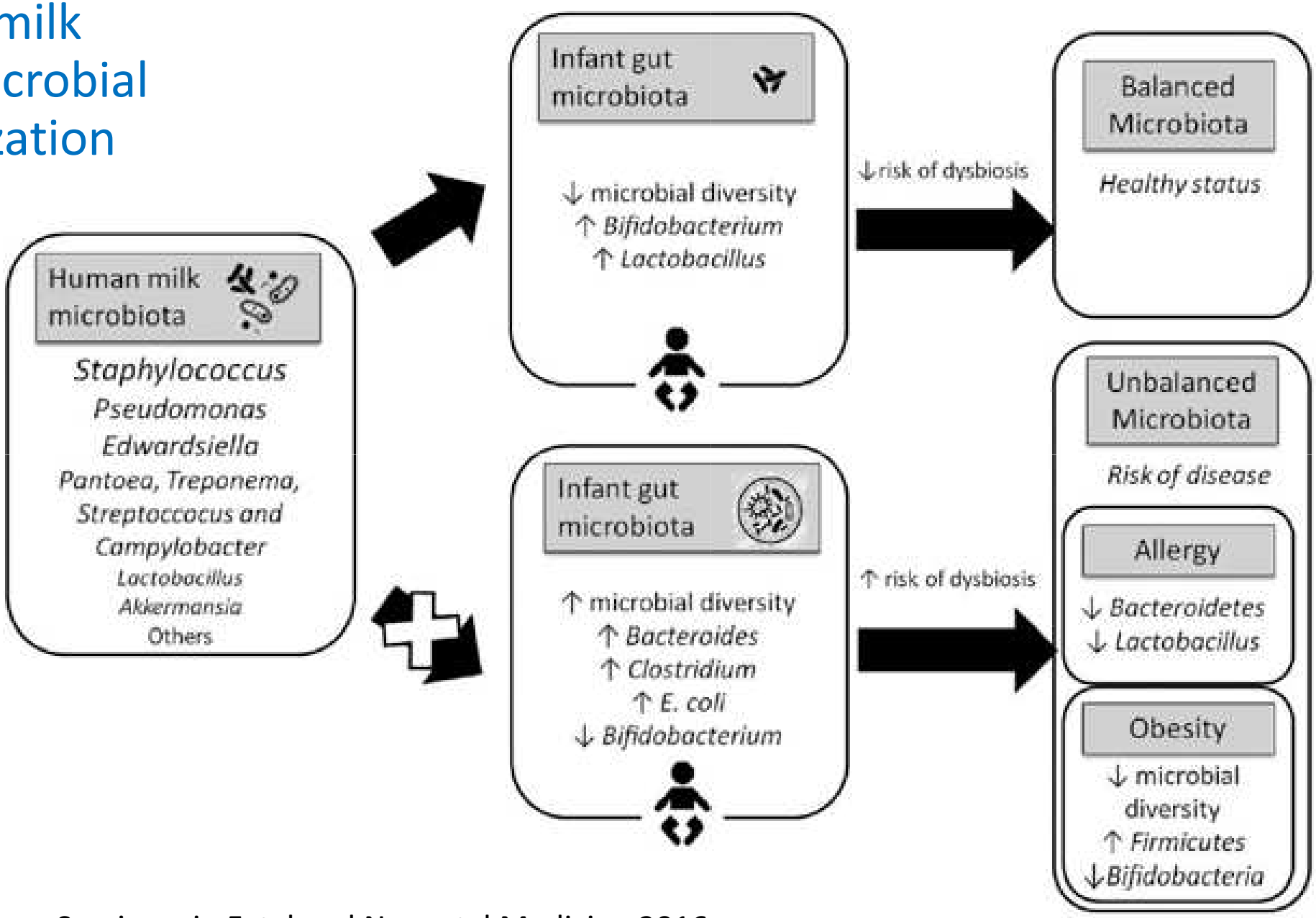


ez-Gallego Seminars in Fetal and Neonatal Medicine 2016

Breastmilk is not sterile

- Bacteria generally isolated in breastmilk of healthy women include:
  - *Staphylococcus*
  - *Streptococcus*
  - *Enterococcus*
  - ***Lactobacillus***
  - ***Bifidobacterium***

Composition of breast milk

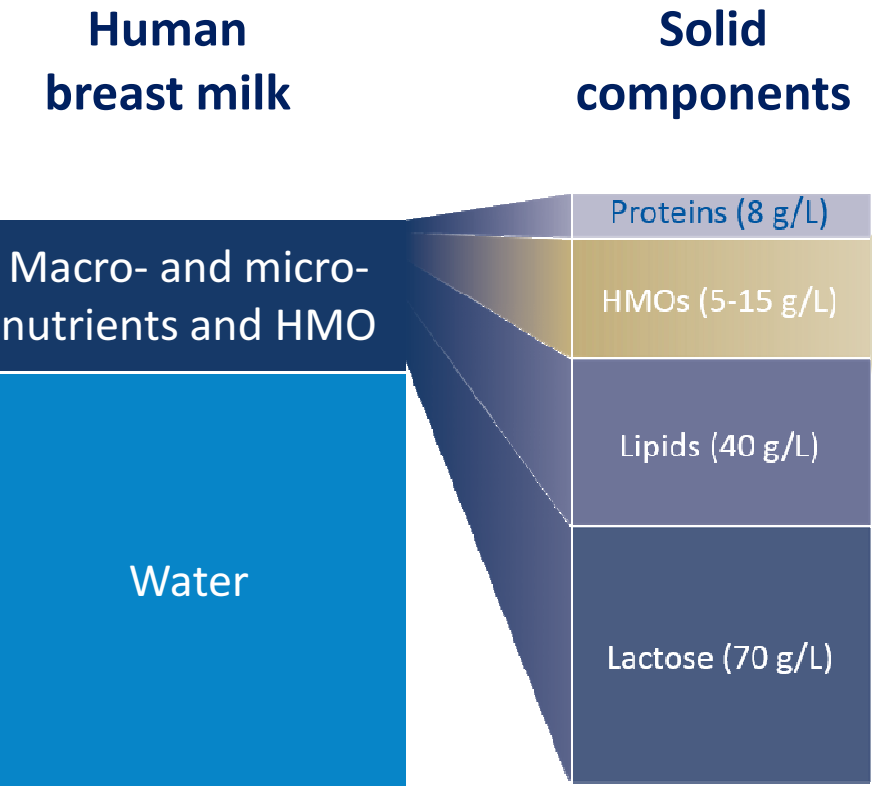
Human breast milk

Macro- and micro-nutrients and HMO

Water

ovic AM, et al. Proc Natl Acad Sci USA. 2011;108(Suppl. 1):4653–8; Austin S, et al. Nutrients 2016;8:pii: E346; Sprenger N, et al. PLoS One 2017;12:e0171814; Kunz C, et al. J Pediatr Gastroenterol Nutr 2017;64:789–98;
e L. Glycobiology 2012;22:1147–1162.

Composition of breast milk



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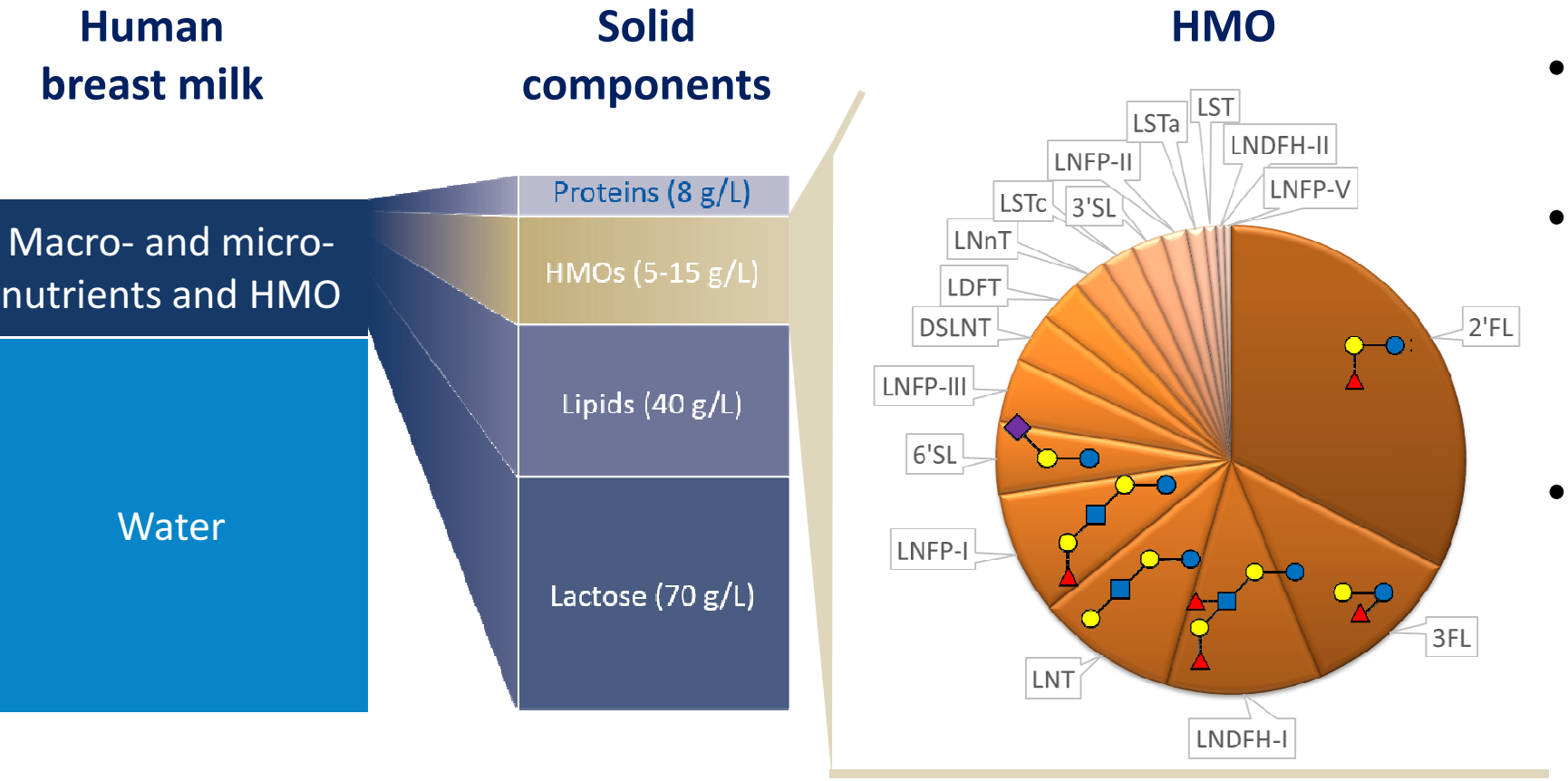
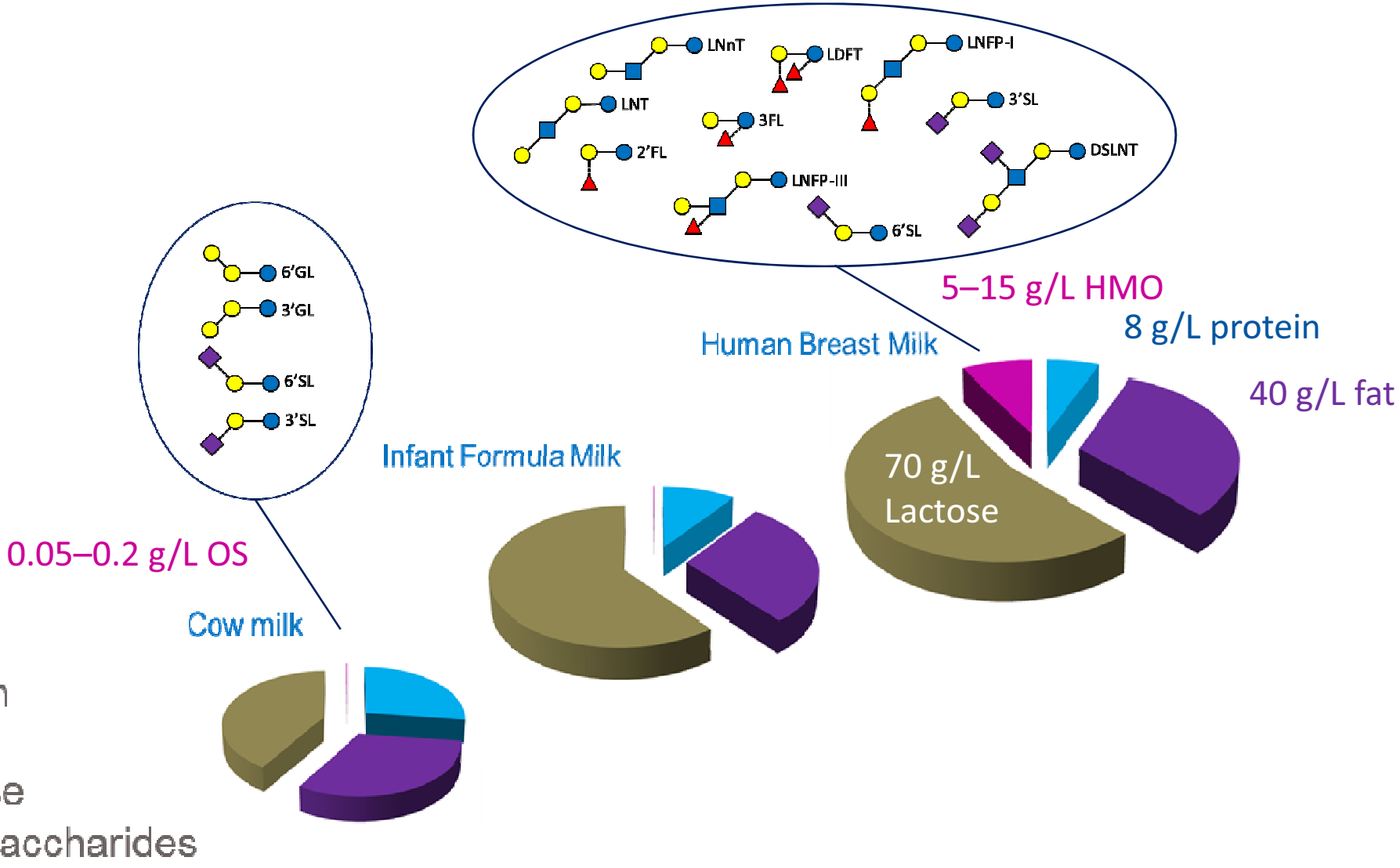


- 5 to 15 g/L in breas milk
- >130 structures described, of which about 15 make up the bulk (>80%)
- Oligosaccharides n generally present i farmed animal milk

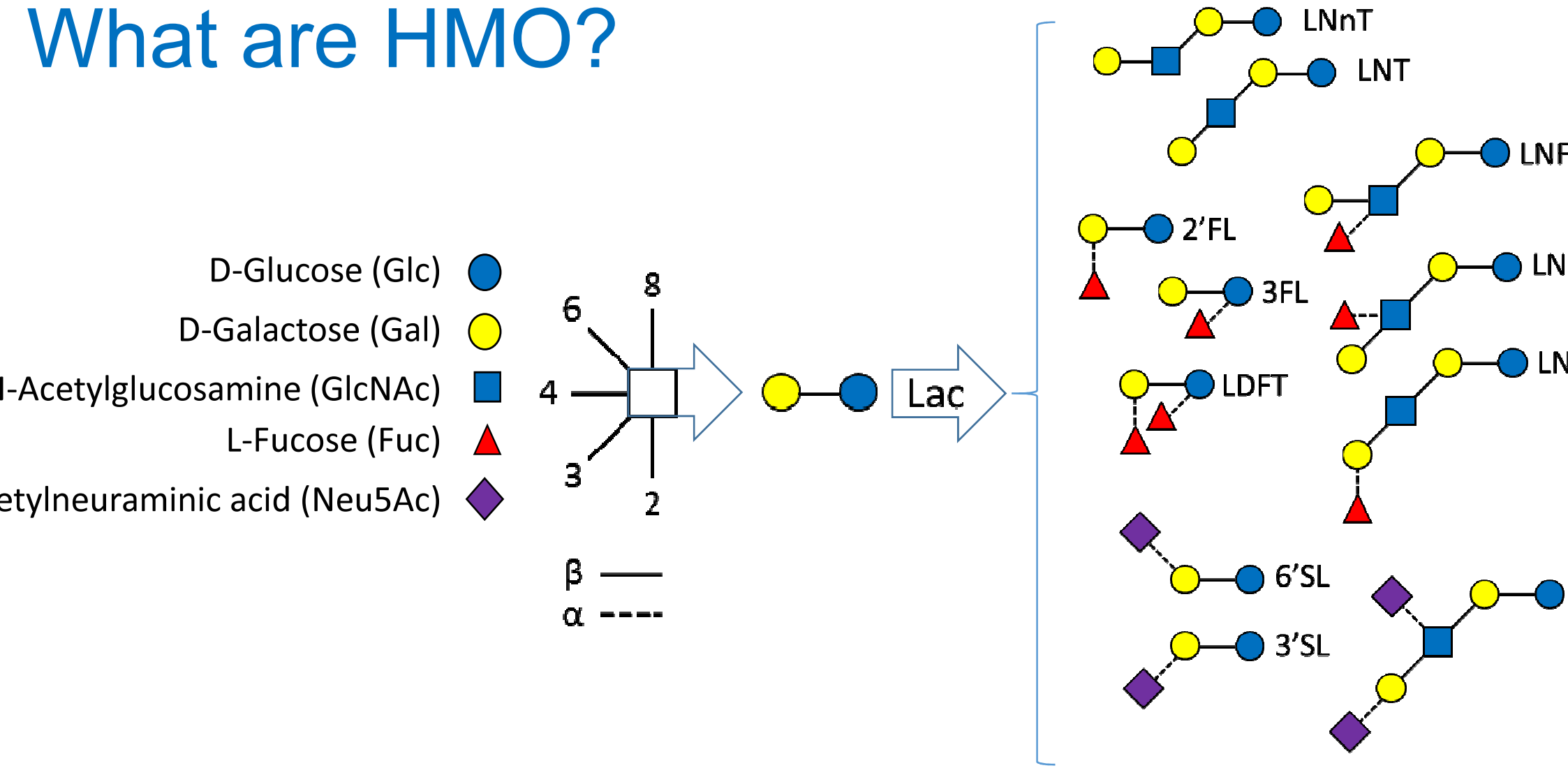
ovic AM, et al. Proc Natl Acad Sci USA. 2011;108(Suppl. 1):4653–8; Austin S, et al. Nutrients 2016;8:pii: E346; Sprenger N, et al. PLoS One 2017;12:e0171814; Kunz C, et al. J Pediatr Gastroenterol Nutr 2017;64:789–98;
e L. Glycobiology 2012;22:1147–1162.

Composition of breast milk, Cow milk and Infant formula



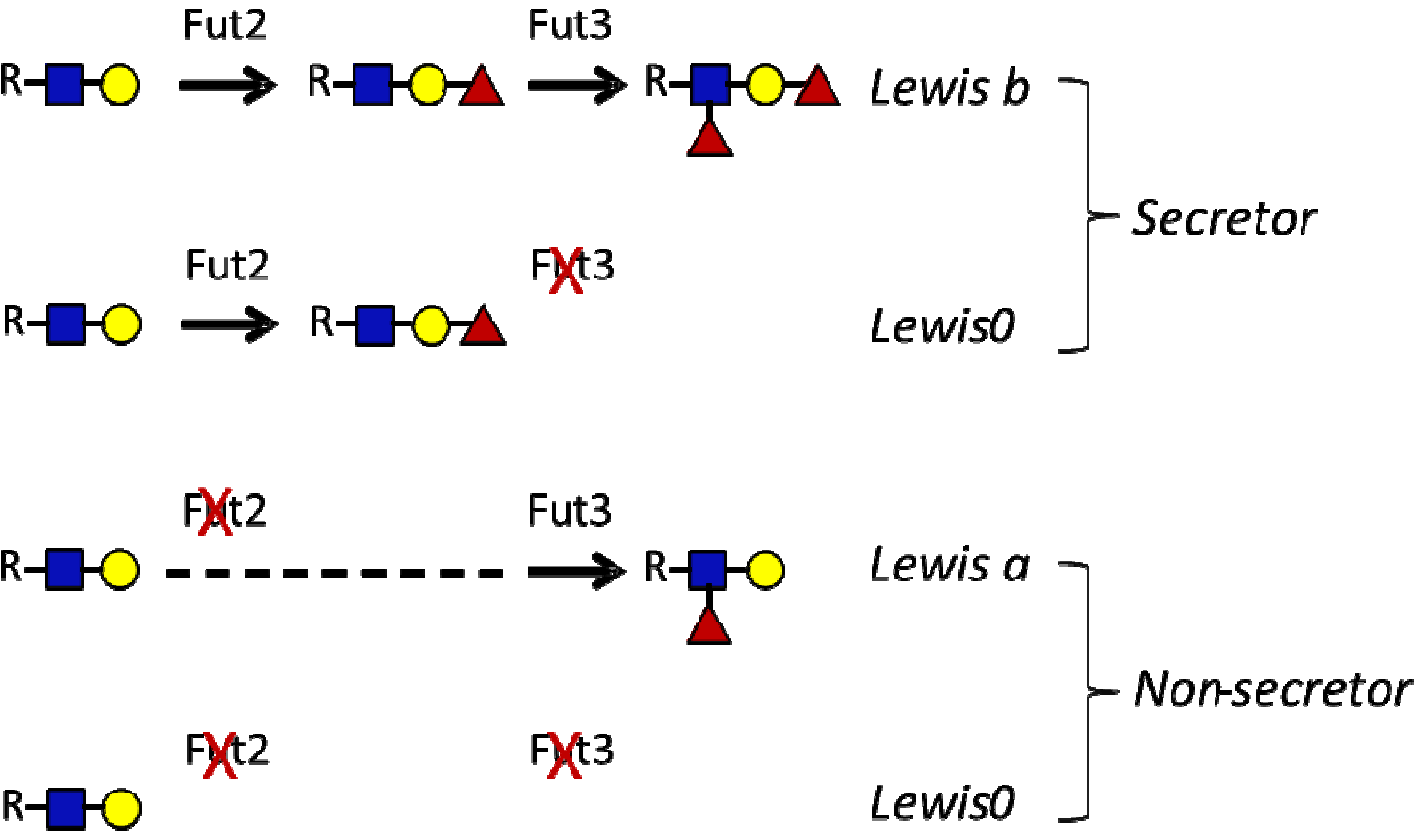
e L. Glycobiology 2012;22:1147–62.

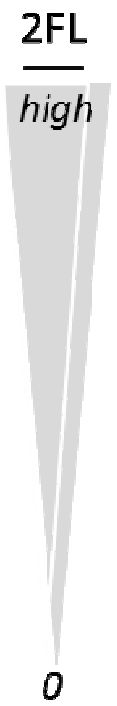
What are HMO?

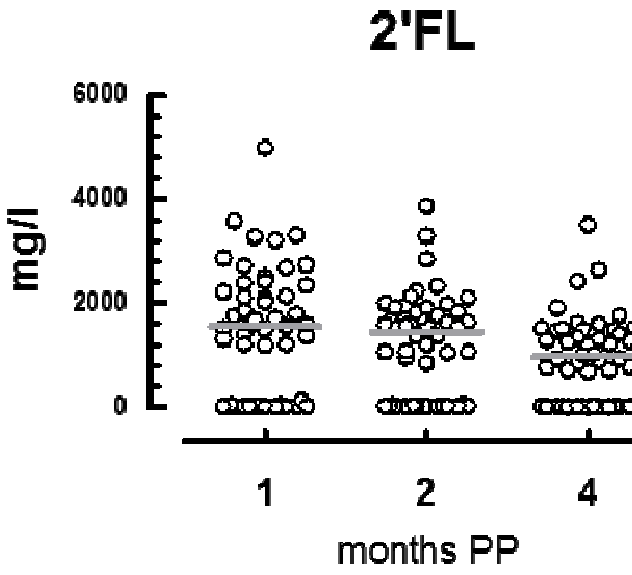


e L. Glycobiology 2012;22:1147–62; Austin S, et al. Nutrients 2016; 8:pii: E346; Sprenger N, et al. PLoS One 2017;12:e0171814.

Maternal genotype determines fucosylated HMOs







Lewis et al. Microbiome (2015) 3:13
DOI 10.1186/s40168-015-0071-z

Microbiome

RESEARCH Open Access

Maternal fucosyltransferase 2 status affects the gut bifidobacterial communities of breastfed infants

Zachery T Lewis[1,4], Sarah M Totten[2,4], Jennifer T Smilowitz[1,4], Mina Popovic[5], Evan Parker[2], Danielle G Lemay[6], Maxwell L Van Tassell[7], Michael J Miller[7], Yong-Su Jin[7], J Bruce German[1,4], Carlito B Lebrilla[2,4] and David A Mills[1,3,4*]

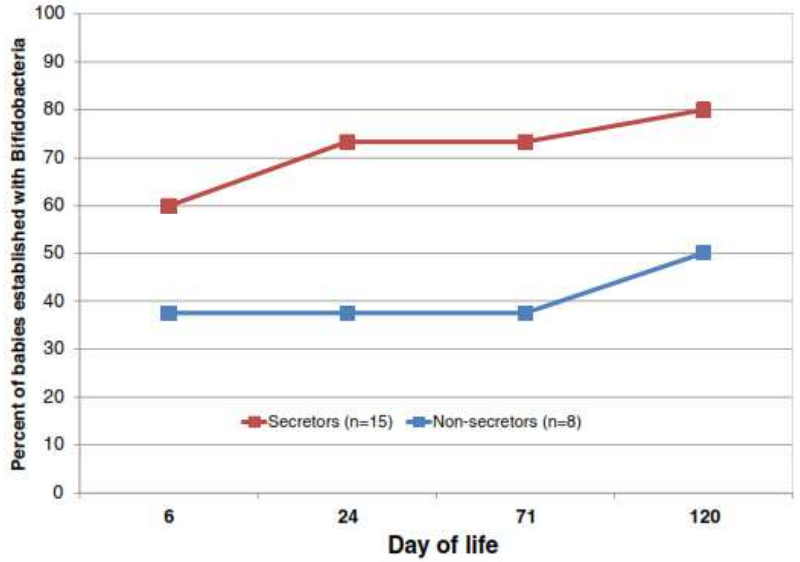


Figure 5 Percentage of infants with high bifidobacteria over time. Based on when each qualifying infant crossed the cutoff point of $10^{8.5}$ bifidobacterial genome equivalents/gram feces. Infants qualified for this analysis by having the appropriate time points available to know when they are first established with bifidobacteria; for example, if the day 6 sample is missing, it is impossible to know if the infant was established at that time or not, and thus, that infant was excluded from this analysis.

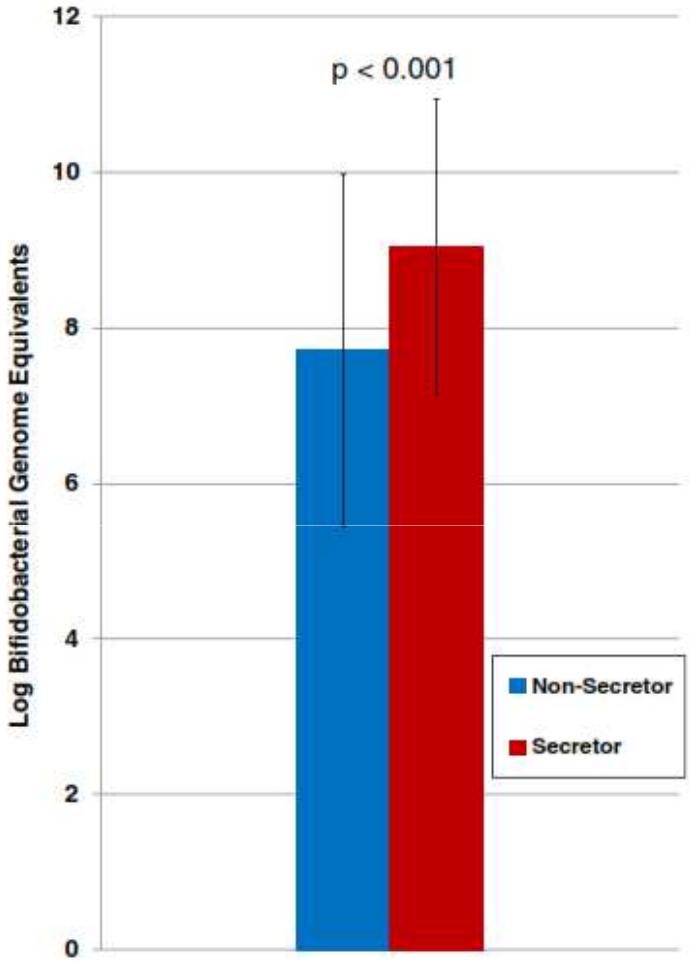


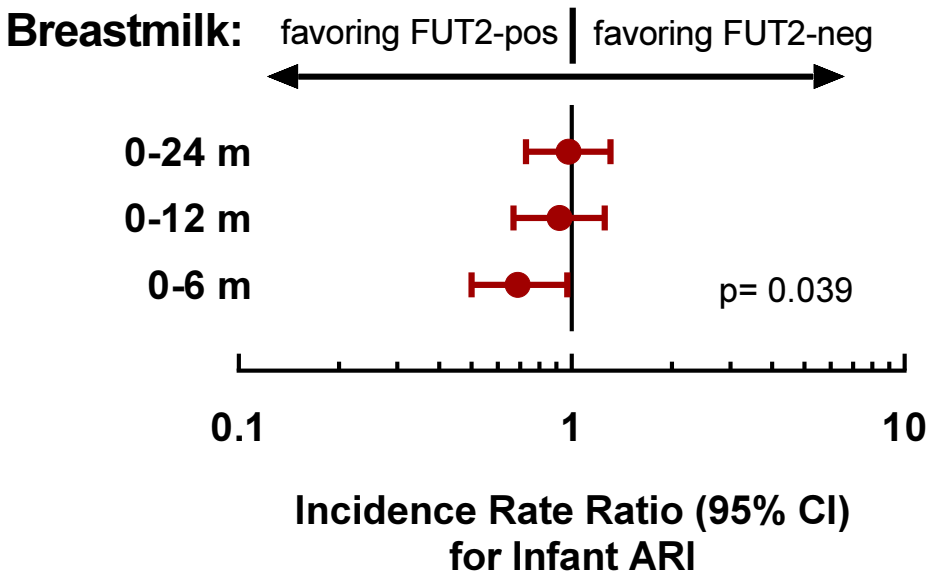
Figure 3 Average absolute levels of bifidobacteria in secretor *versus* non-secretor-fed infants (all samples of each secretor status averaged together). The one-tailed type three *t*-test *p* value was <0.001.

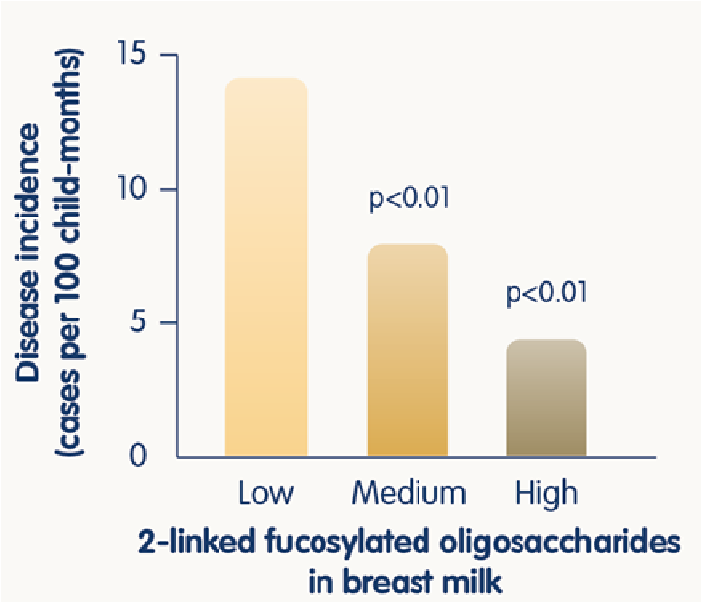
Insight on HMO from observational clinical studies

2’Fucosyl-HMO in breastmilk is related to:

- Lower risk for ARI during the predominant breastfeeding period

- Lower incidence of infectious diarrhea in infants at 9 months
- Lower morbidity at 4 months



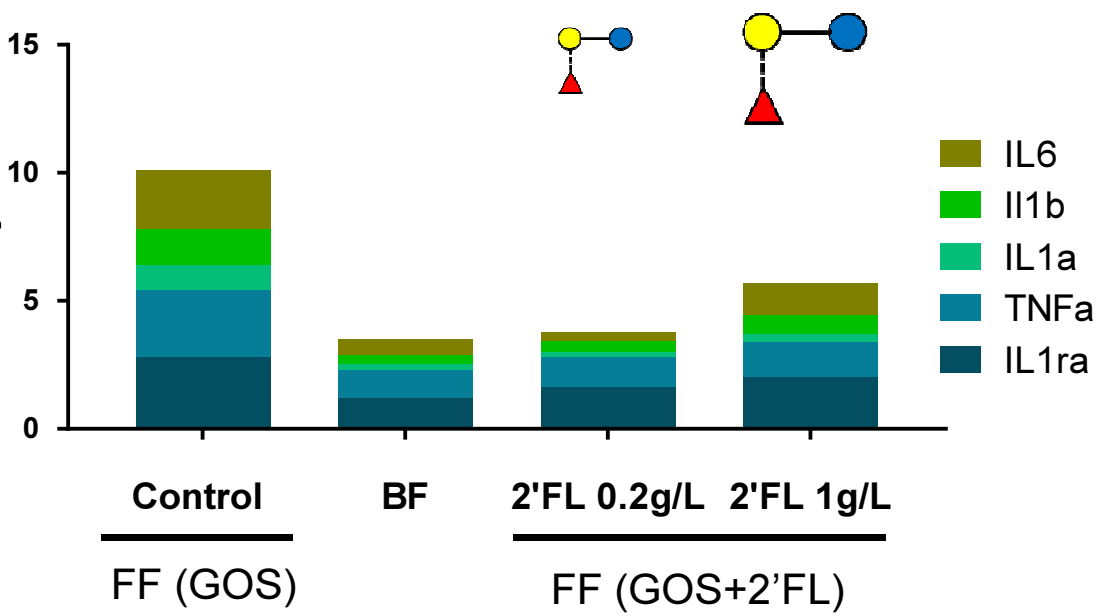


acute respiratory infections.

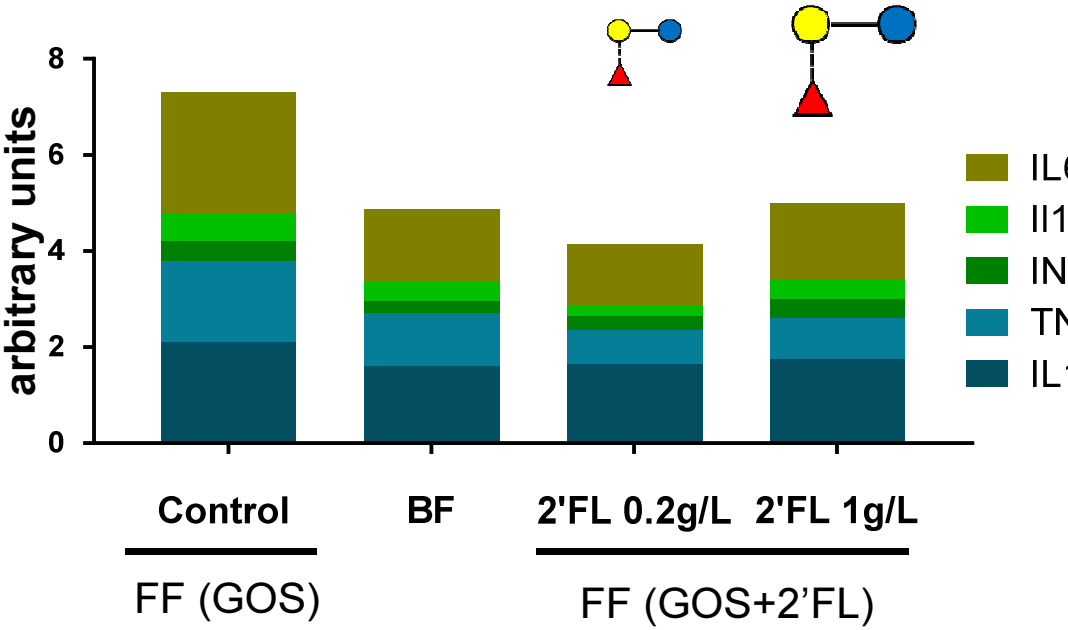
, Sakwinska, et al. NRC, unpublished results; Morrow AL, et al J Pediatr 2004;145:297–303; Davis JC, et al. Sci Rep 2017;7:40466; Stepans MB, et al. Breastfeed Med 2006;1:207–15.

RCT infants fed a starter formula with 2’FL show a plasma immune marker profile similar to breastfed infants

Plasma baseline immune marker profile in 6-week-old infants



Cytokine production by RSV-stimulated PBMCs from 6-week-old infants, *ex vivo*



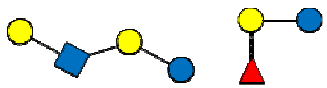
(n=30–40/group)

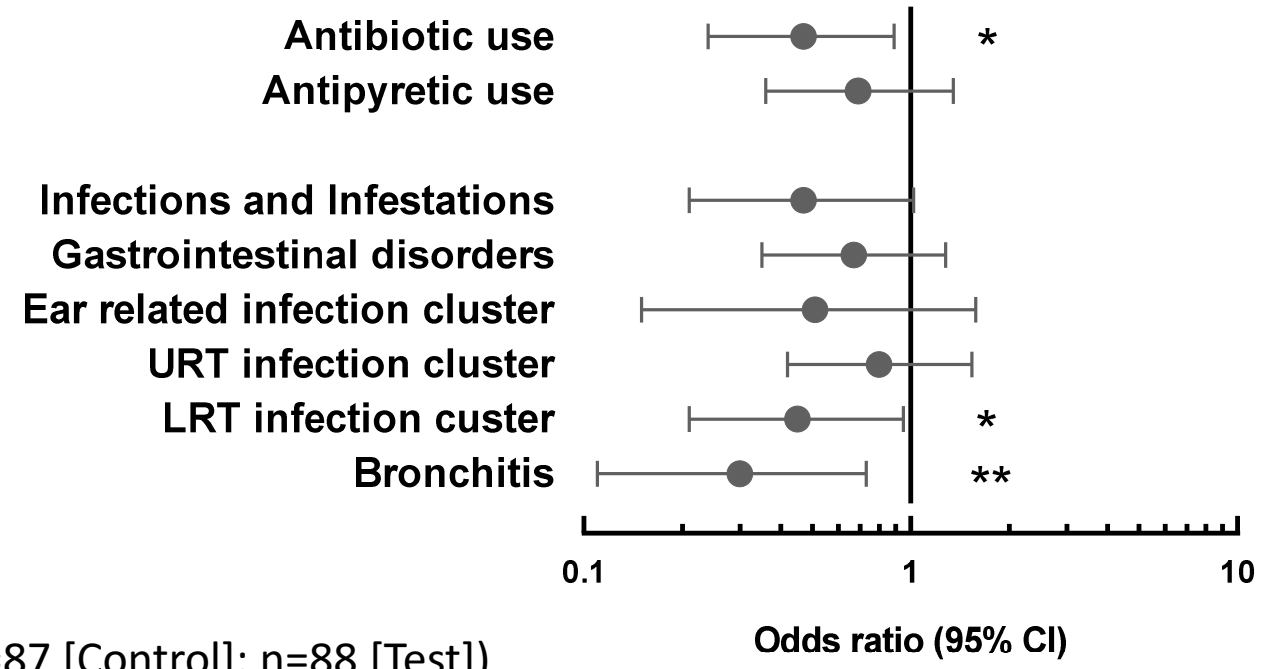
reastfed; FF, formula fed; GOS, galacto-oligosaccharides; RSV, respiratory syncytial virus.
oted from Goehring K, et al. J Nutr 2016;146:2559–66.

RCT infants fed a starter formula with HMO (2'FL & LnNT) have a lower risk for reported lower respiratory tract infections and antibiotics use during first year of life

Reduced morbidity and medication use

- Fewer reports of infections and infestations, lower respiratory tract illnesses, notably bronchitis through 12 months
- Less frequent antibiotics use through 12 months
- Less frequent antipyretics use through 4 months





(n=87 [Control]; n=88 [Test])

oted from Puccio G, et al. J Pediatr Gastroenterol Nutr 2017;64:624–31.

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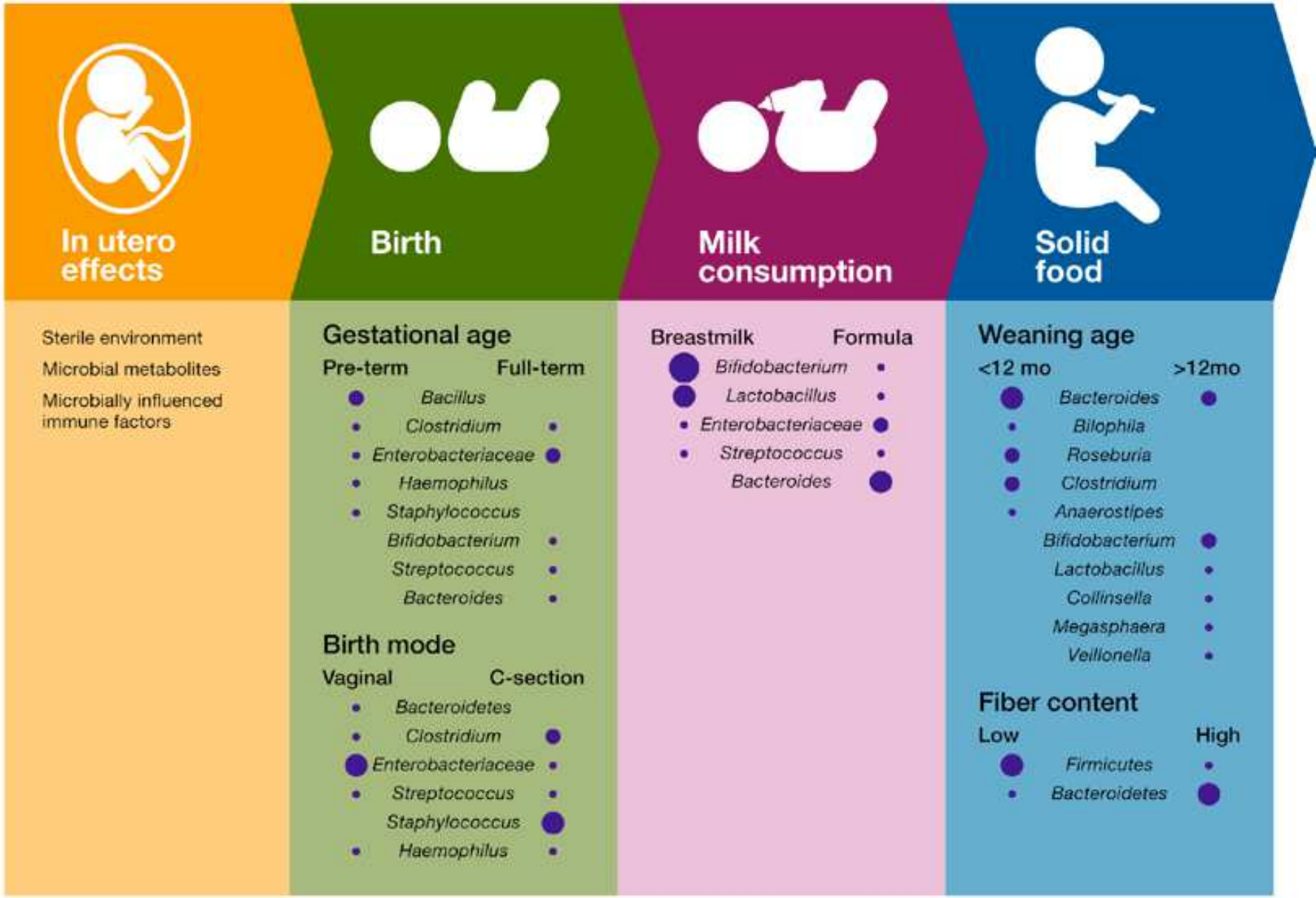


st-Lapointe et al, Frontiers in Immunology, 2017

“Modern” Lifestyle Has Decreased Exposure to Bacteria

- Cesarean birth
- No breastfeeding
- Sanitized food supply - including Infant formula
- Urban life
- Antibiotics

Lower Oral microbial exposure

Altered Intestinal microbiota: Dysbiosis

Inadequate immune response

Rautava S., et al. JPGN 2004;38:378-388
Rook GA. Immunol Today 1998;19:113-116

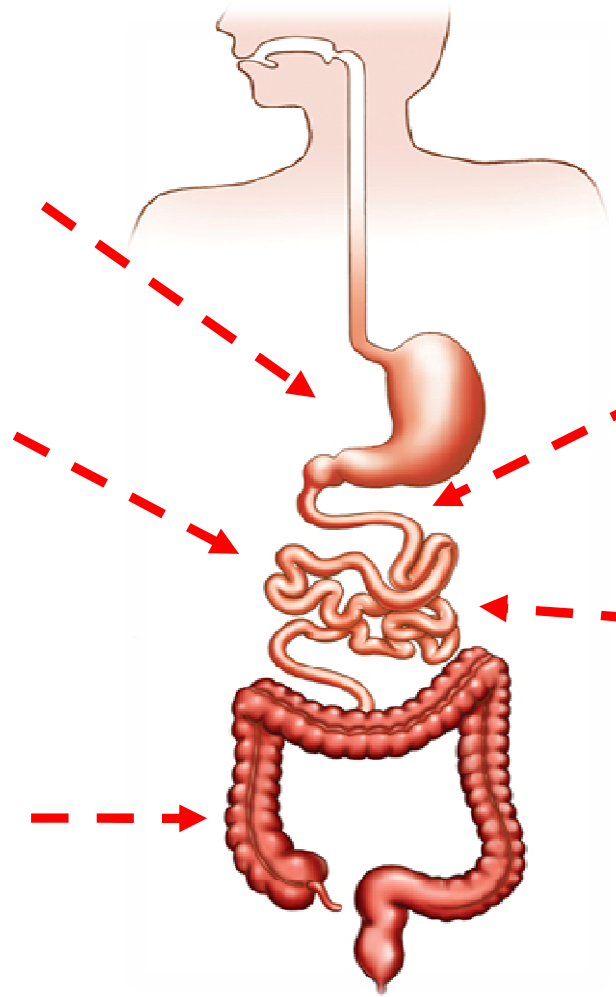
Bacteria in the GI Tract: Complex Ecosystem

Resident and Ingested

Stomach
10^4 CFU/g
Candida albicans
Helicobacter pylori
Lactobacillus
Streptococcus

Jejunum
10^5-10^7 CFU/g
Bacteroides
Candida albicans
Lactobacillus
Streptococcus

Colon
10^{10}-10^{11} CFU/g
Bacteroides
Bacillus
Bifidobacterium
Clostridium
Enterococcus
Eubacterium
Fusobacterium
Peptostreptococcus
Ruminococcus
Streptococcus



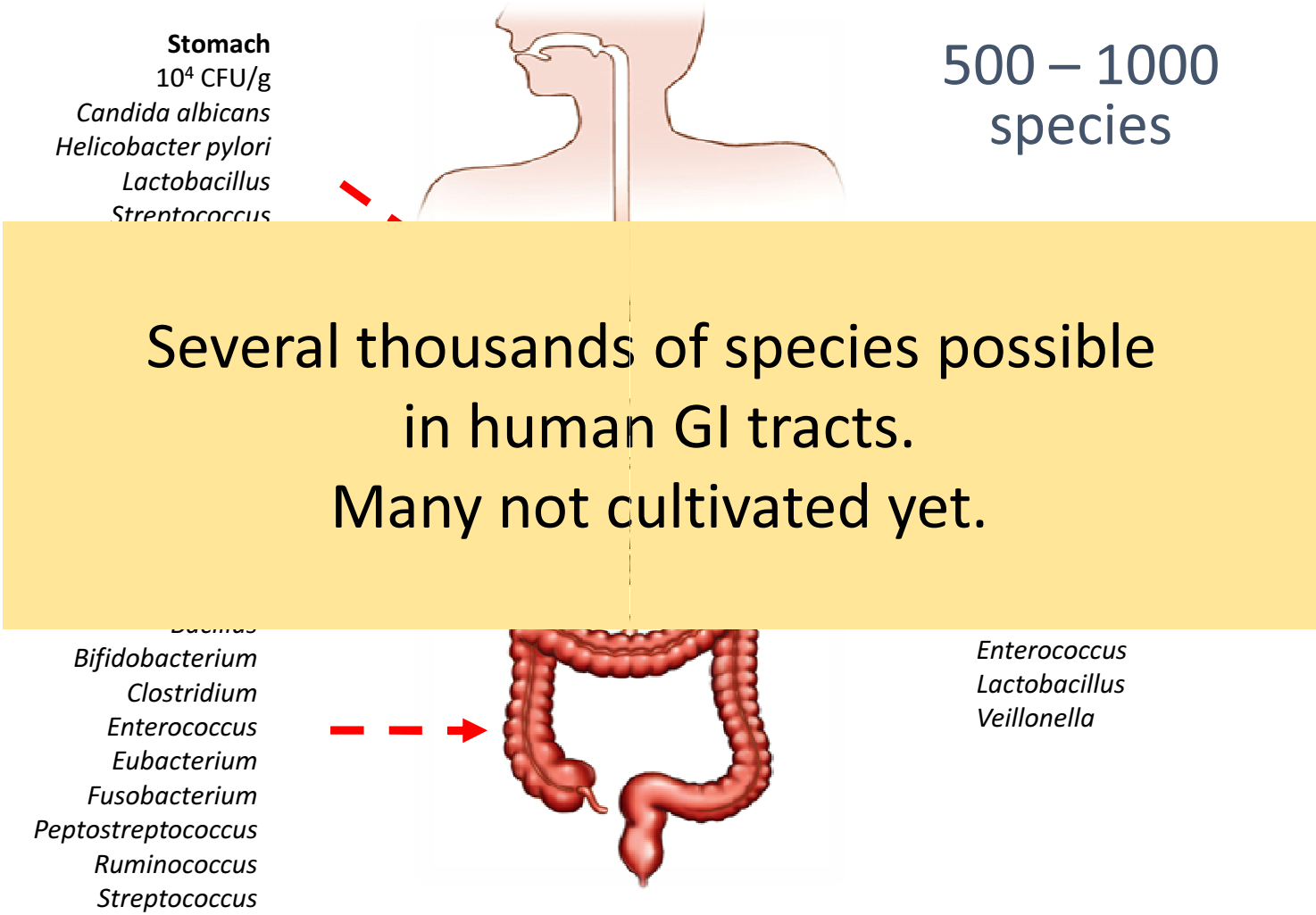
500 – 1000 species

Duodenum
10^3-10^4 CFU/g
Bacteroides
Candida albicans
Lactobacillus
Streptococcus

Ileum
10^7-10^8 CFU/g
Bacteroides
Clostridium
Enterobacteriaceae
Enterococcus
Lactobacillus
Veillonella

Bacteria in the GI Tract: Complex Ecosystem

Resident and Ingested



Staphylococcus, Clostridium spp
Listeria
Lactobacilli, Streptococcus, Leuconostoc, Enterococcus

Bacteoides fragilis, spp
Prevotella

Enterobacteriaceae, Rickettsidae
Neisseriales
Vibrionaceae, Pseudomonadaceae.
Salmonella, Yersinia, Vibrio, Pseudomonas, Escherichia, Shigella
Campylobacter, Helicobacter

Main human GI microbial species

Bacilli
- Bacillales [1]
- Lactobacillales [2]
- Mollicutes
- Erysipelotrichia
- Erysipelotrichales

Clostridia
- Clostridiales [3]
- Halanaerobiales
- Natranaerobiales
- Thermoanaerobacterales
- Negativicutes
- Selenomonadales

Thermolithobacteria

Staphylococcus, Clostridium spp Listeria Lactobacilli, Streptococcus, Leuconostoc, Enterococcus

Bacteroidia
- Bacteroidales [1]

Balneolia
- Balneolales

"Chitinophagia"
- "Chitinophagales"

Cytophagia
- Cytophagales

Flavobacteriia
- Flavobacteriales

Rhodothermia
- Rhodothermales

Sphingobacteria
- Sphingobacteriales

Bacteoides fragilis, spp Prevotella

Alphaproteobacteria [1]
Betaproteobacteria [2]
Hydrogenophilalia
Gammaproteobacterian[3]
Acidithiobacilli
Deltaproteobacteria
Epsilonproteobacteria [4]
Oligoflexia

Enterobacteriaceae, Ricketsidae Neisseriales Vibrionaceae, Pseudomonadaceae. Salmonella, Yersinia, Vibrio, Pseudomonas, Escherichia, Shigella Campylobacter, Helicobacter

Rubrobacteria
Thermoleophilia
Coriobacteriia
Acidimicrobiia
Nitriliruptoria
Actinobacteria
- Actinobacteriales [1]

Bifidobacteria spp

Main human GI microbial species

Firmicutes

Bacilli
- Bacillales [1]
- Lactobacillales [2]
- Mollicutes
- Erysipelotrichia
- Erysipelotrichales

Clostridia
- Clostridiales [3]
- Halanaerobiales
- Natranaerobiales
- Thermoanaerobacterales
- Negativicutes
- Selenomonadales

Thermolithobacteria

Staphylococcus, Clostridium spp Listeria Lactobacilli, Streptococcus, Leuconostoc, Enterococcus

Bacteroidetes

Bacteroidia
- Bacteroidales [1]

Balneolia
- Balneolales

"Chitinophagia"
- "Chitinophagales"

Cytophagia
- Cytophagales

Flavobacteriia
- Flavobacteriales

Rhodothermia
- Rhodothermales

Sphingobacteria
- Sphingobacteriales

Bacteoides fragilis, spp Prevotella

Proteobacteria

Alphaproteobacteria [1]
Betaproteobacteria [2]
Hydrogenophilalia
Gammaproteobacterian[3]
Acidithiobacilli
Deltaproteobacteria
Epsilonproteobacteria [4]
Oligoflexia

Enterobacteriaceae, Rickettsidae Neisseriales Vibrionaceae, Pseudomonadaceae. Salmonella, Yersinia, Vibrio, Pseudomonas, Escherichia, Shigella Campylobacter, Helicobacter

Actinobacte

Rubrobacteria
Thermoleophilia
Coriobacteriia
Acidimicrobiia
Nitriliruptoria
Actinobacteria
- Actinobacteriales [1]

Bifidobacteria spp

Main human GI microbial species

16S ribosomal RNA – based phylogeny

16S rRNA gene

- Present in almost all bacteria and archaea
- Its function has been preserved over time
- It consists of both conserved and variable regions
- The conserved region makes universal amplification possible
- Sequencing the variable regions allows discrimination between specific different microorganisms

- Described from an initial group of 11 bacterial phyla in 1987,
- As of February 2012, 2 million 16S rRNA sequences and 35 phyla described

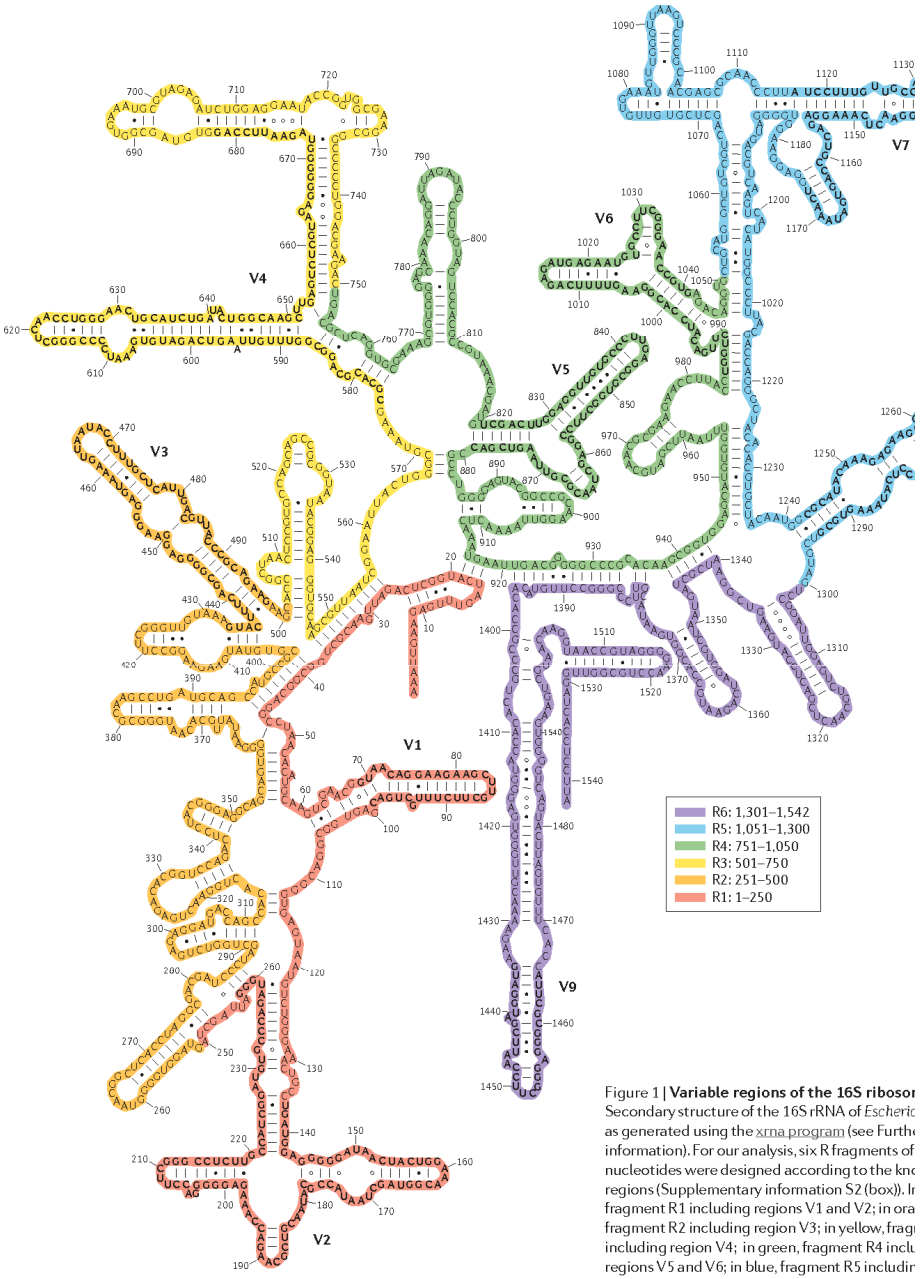
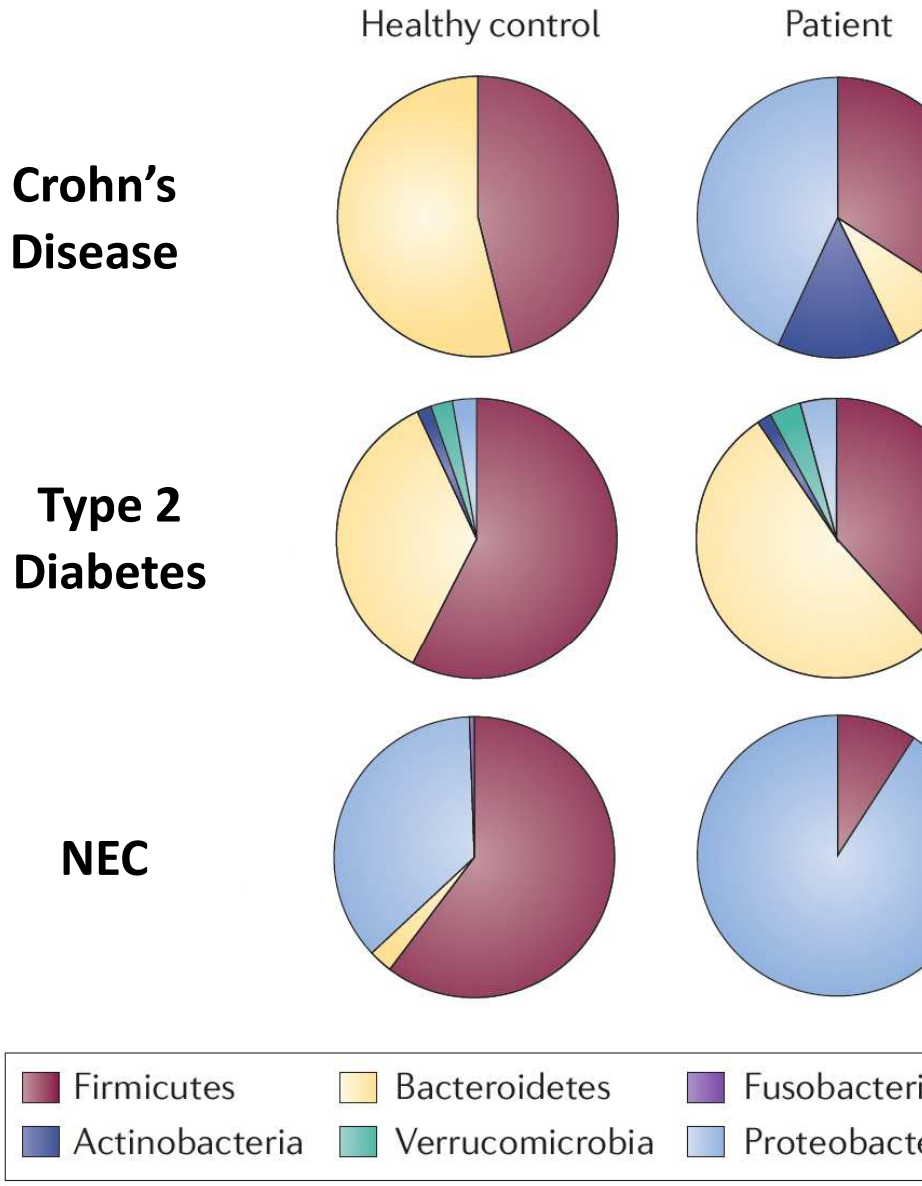


Figure 1 | **Variable regions of the 16S riboso**
Secondary structure of the 16S rRNA of *Escheric*
as generated using the xrna program (see Furth
information). For our analysis, six R fragments of
nucleotides were designed according to the kn
regions (Supplementary information S2 (box)). I
fragment R1 including regions V1 and V2; in ora
fragment R2 including region V3; in yellow, frag
including region V4; in green, fragment R4 inclu
regions V5 and V6; in blue, fragment R5 includi

Dysbiosis:
Altered Microbiota associated with acute and chronic diseases

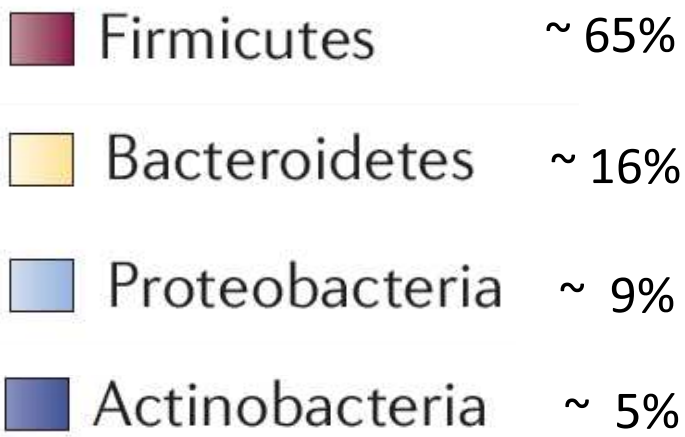


wski KM, Nature Immunology, 2011 12:6

Spor et al Nature Reviews - Microbiology

lain human GI
icrobial phyla

Adult Microbiome



t al Nature Reviews – Microbiology, 2011

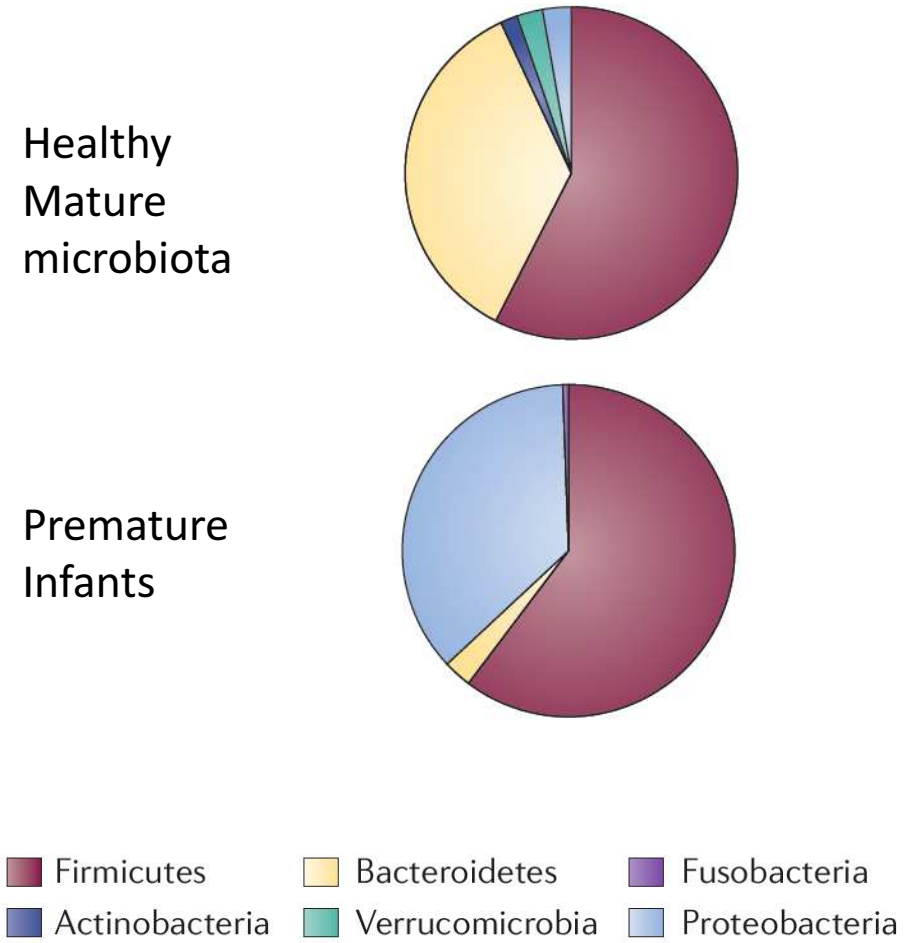
lain human GI
icrobial phyla

Adult Microbiome

Firmicutes	~ 65%
Bacteroidetes	~ 16%
Proteobacteria	~ 9%
Actinobacteria	~ 5%

Healthy Mature microbiota

Premature Infants



t al Nature Reviews – Microbiology, 2011

Microbiota & obesity

frontiers in Microbiology

ORIGINAL RESEARCH

ut Microbiota Markers in Obese dolescent and Adult Patients: ge-Dependent Differential Patterns

*erica Del Chierico[1], Francesca Abbatini[2], Alessandra Russo[1], Andrea Quagliariello[1], a Reddel[1], Danila Capoccia[3], Romina Caccamo[4], Stefano Ginanni Corradini[5], erio Nobili[6,7], Francesco De Peppo[4], Bruno Dallapiccola[8], Frida Leonetti[3], nfranco Silecchia[2] and Lorenza Putignani[1,9]**

Phyla

- ↑ Firmicutes
- ↓ Bactreroidetes

Genus / species

Faecalibacyterium prausnitzii

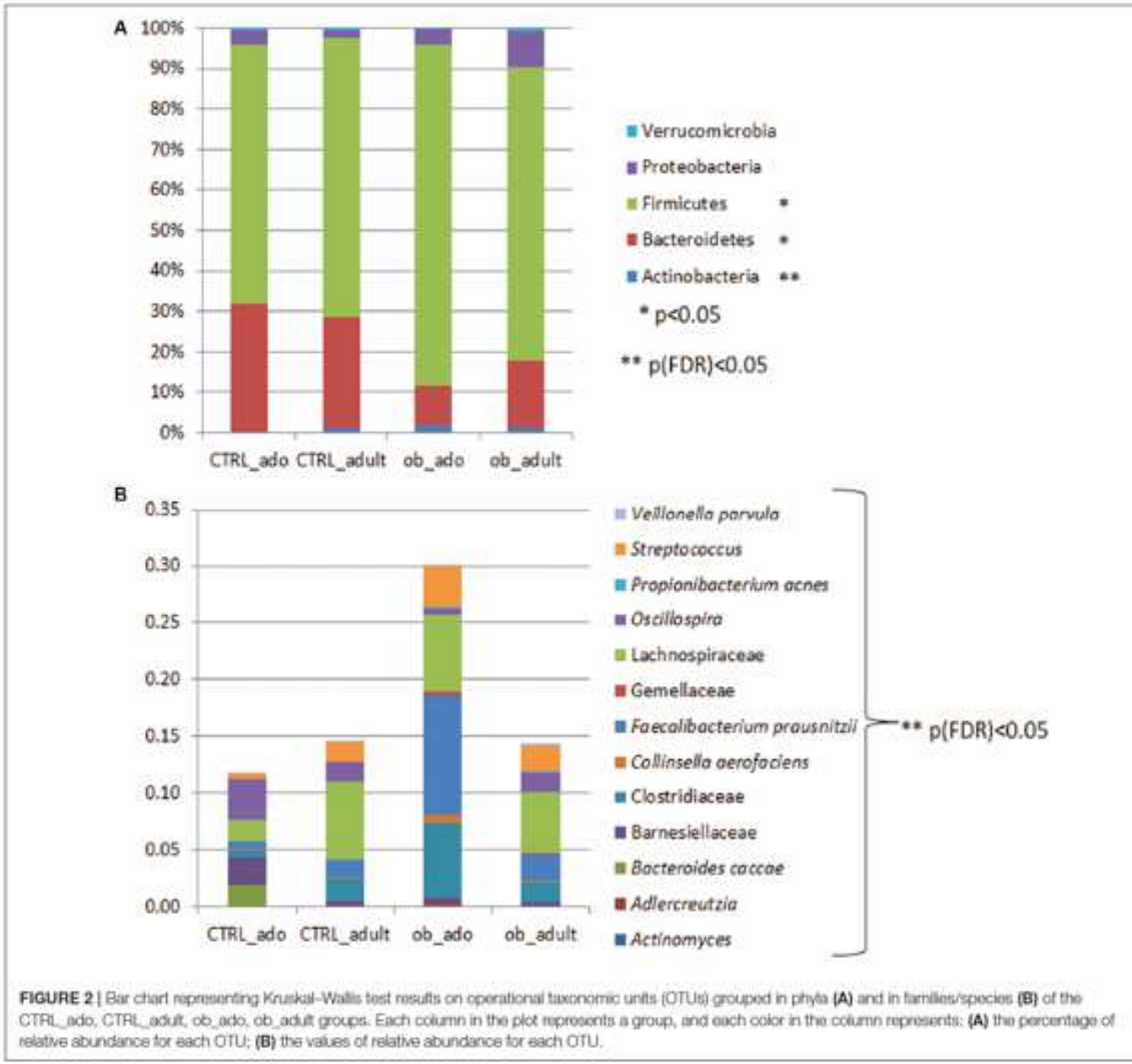
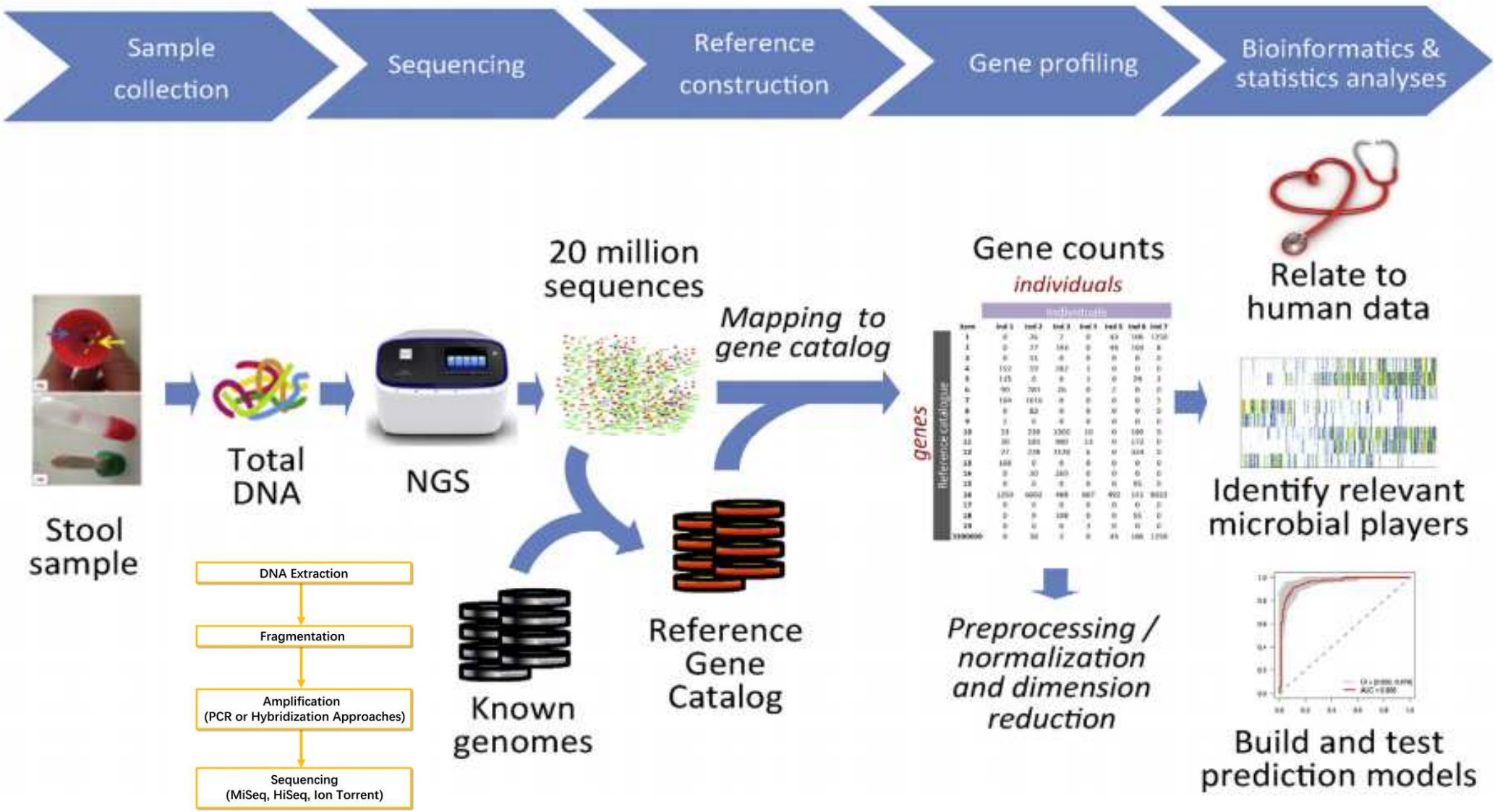


FIGURE 2 | Bar chart representing Kruskal–Wallis test results on operational taxonomic units (OTUs) grouped in phyla **(A)** and in families/species **(B)** of the CTRL_ado, CTRL_adult, ob_ado, ob_adult groups. Each column in the plot represents a group, and each color in the column represents: **(A)** the percentage of relative abundance for each OTU; **(B)** the values of relative abundance for each OTU.

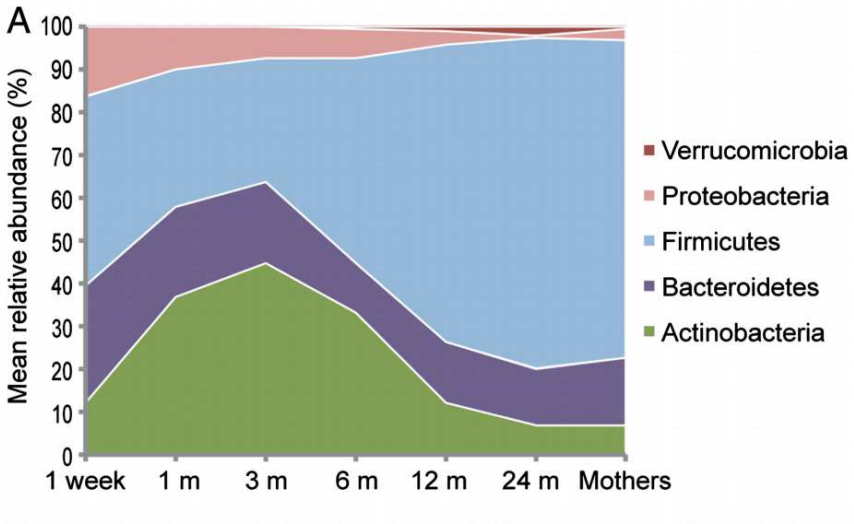
hiericco et all 2018

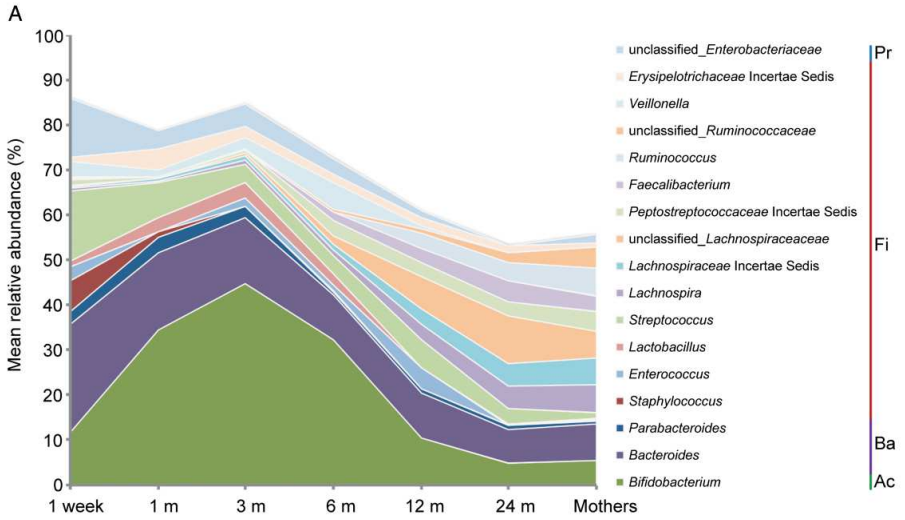


neration Sequencing has changed the way we look at the mocrobiome
Ekrlich S, Compte Rendus Biologies, 2016
i F et al, NGS tools, 2016

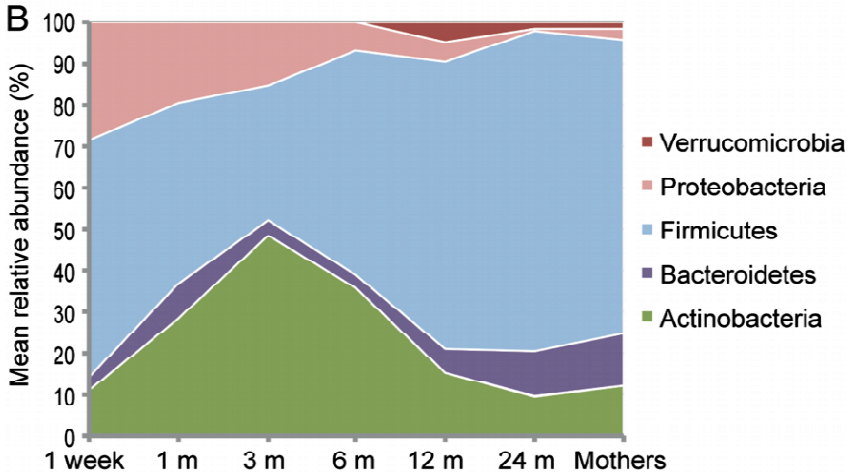
ean relative abundance

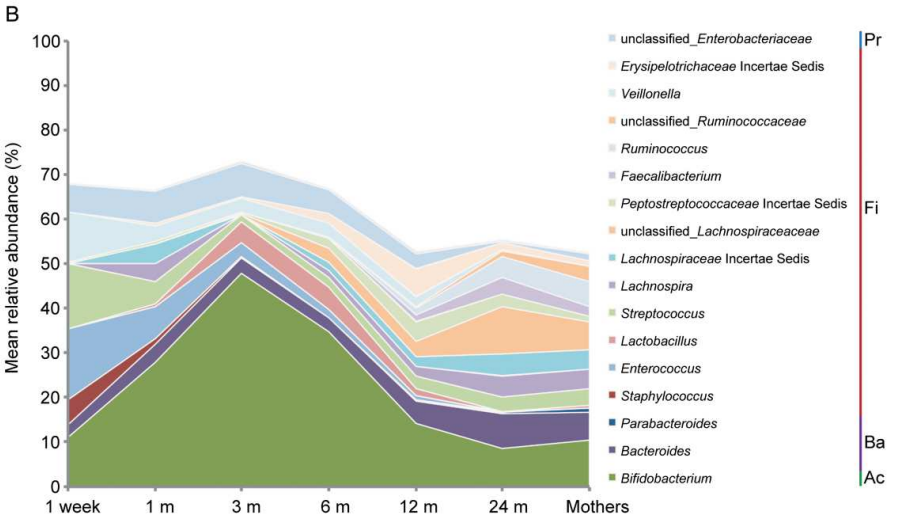
Vaginally delivered
n = 15





C section delivered
n = 9





Phylum level

Genus level

n et al, Gut, 2013

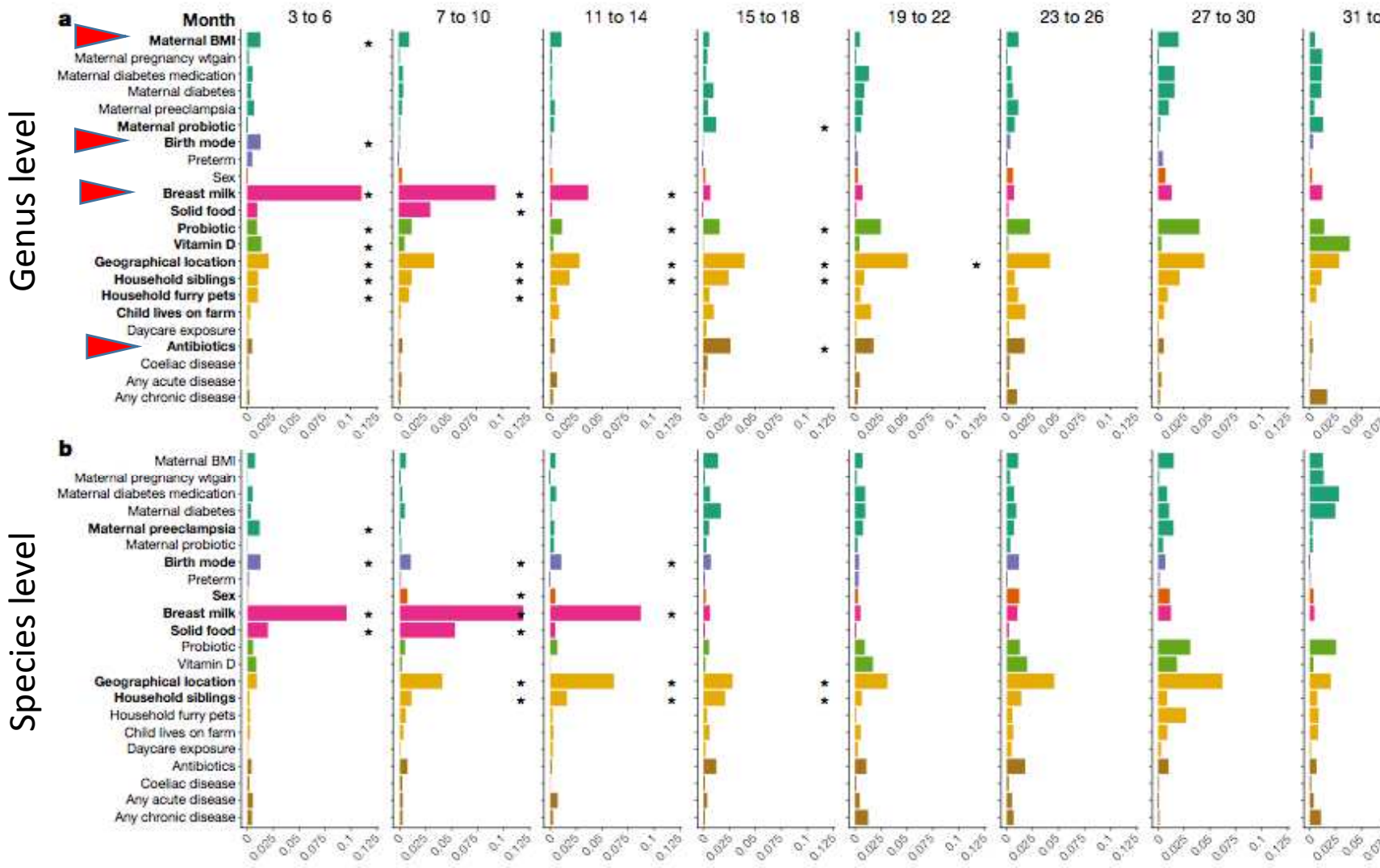
The TEDDY Study
Germany, Sweden,
and, USA


ETTER

OPEN

https://doi.org/10.1038/s41586-018-0617-x

mporal development of the gut microbiome in
ly childhood from the TEDDY study



Amount of variance explained by each covariate

N = 987 children, >10,000 samples

t C et al, Nature, 2018

Can we modify the intestinal microbiota?

The GI Microbiota is a stable ecosystem

– difficult to disrupt

- Diet
- Antibiotics
- Oral ingestion of bacteria (probiotics)
- Oral ingestion of bacterial substrates (prebiotics)
- Replacement of microbiota (fecal transplantation

THE LANCET

Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus

Jose M Saavedra, Nancy A Bauman, Irene Oung, Jay A Perman, Robert H Yolken

Summary

Acute diarrhoea is a serious cause of infant morbidity and mortality, and the development of preventive measures remains an important goal. Bifidobacteria (which constitute the predominant intestinal flora of breastfed infants), as well as other lactic-acid-producing organisms such as *Streptococcus thermophilus*, are thought to have a protective effect against acute diarrhoeal disease. However, their efficacy has not been assessed in controlled trials.

In a double-blind, placebo-controlled trial, infants aged 5–24 months who were admitted to a chronic medical care hospital were randomised to receive a standard infant formula or the same formula supplemented with *Bifidobacterium bifidum* and *S thermophilus*. Patients were evaluated daily for occurrence of diarrhoea, and faecal samples, obtained weekly, were analysed for rotavirus antigen by enzyme immunoassay. Faecal samples were also obtained during an episode of diarrhoea for virological and bacteriological analyses. 55 subjects were evaluated

Introduction

Acute diarrhoea is a major cause of infant mortality in developing countries.[1,2] Furthermore, nosocomially acquired diarrhoeal disease in infants can lead to a prolonged stay in hospital and to increased medical costs. Although there are many microbial agents associated with gastroenteritis in this age group, rotavirus is the most important cause of the condition in infants admitted to hospital in the USA and in many other countries.[3] The development of effective methods to prevent acute gastroenteritis remains an important goal for infant health.[4]

The replication of pathogenic organisms within the gastrointestinal tract is determined by various microbial and host factors. One such factor is the composition of non-pathogenic intestinal flora. For example, the anaerobic bacteria of the genus *Bifidobacterium* constitute the predominant colonic flora of breastfed infants.[5] Bifidobacteria are thought to exert some of the protective effect against diarrhoea associated with breastfeeding.[6] Additionally, in laboratory animals bifidobacteria reduce

ationale

- Bifidobacteria, the most abundant genus in breastfed infants
- Technology allowed the growth and production of adequate cultures
- Potential for competing with pathogenic bacteria

THE LANCET

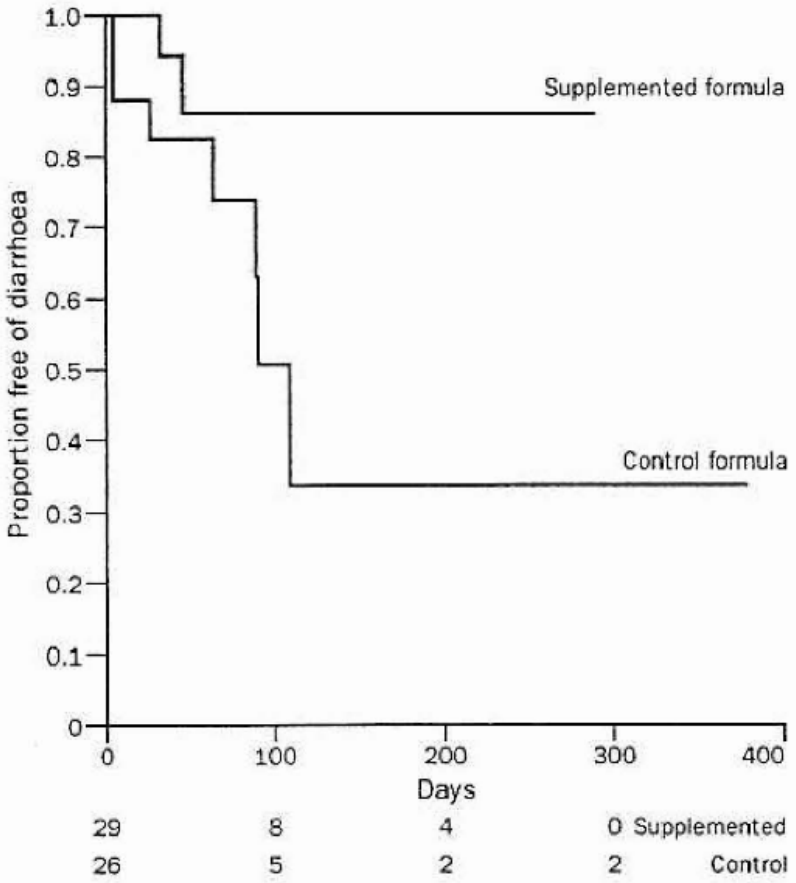


Figure: **Cumulative incidence of diarrhoea in infants receiving formula supplemented with *Bifidobacterium bifidum* and *Streptococcus thermophilus* (supplemented) and the same formula without these bacteria (control)**

October 1994

2 Healthful Bacteria Are Proved To Ward Off Diarrhea in Infants

By WARREN E. LEARY
Special to The New York Times

WASHINGTON, Oct. 13 — Bacteria common to breast milk and yogurt can greatly reduce the risk of infants developing diarrhea, indicating that such "good germs" can be added to foods to attack wide health problem, said today.

Scientists at Johns Hopkins Children's Center in Baltimore had conducted the first study to prove that adding common bacteria to infant formula could cut the risk of developing diarrhea in youngsters; in this study the risk was cut by almost 80 percent.

In a report being published Saturday in The Lancet, a medical journal, researchers said that the bacteria, Bifidobacterium bifidum, which is commonly detected in breast milk and leading some people to add milk to other foods used in schools and day care centers. But he said could not yet make specific recommendations to parents on giving their children yogurt or other sources of the bacteria. More research is needed, he said, on

Good germs help keep babies well

■ "Breast is best," one scientist says, but introducing certain bacteria to baby formula can help prevent diarrhea.

tion of diarrheal diseases around the world," said Robert Yolken, one of the researchers and a professor of pediatrics at Johns Hopkins University School of Medicine.

The study is to be published in Saturday's issue of *The Lancet*, a

, breast is best," use breast milk d bacteria. "But provide some of ast milk to chil- ve it."

estive system is nies of germs, ng harmoniously and our body. es, however, ag- rish.

t formula devel- opkins research-

ers is an attempt to restore the natural environment.

"Their findings seem logical," said Dr. Warren Andiman, professor of pediatrics at Yale University. "It's not a totally novel idea, but using it in a slightly different way."

A leading physician at the turn of the century advocated treating patients with germs.

Since then, some doctors and nutritionists have promoted yogurt with live bacteria to heal the body, particularly for patients taking antibiotics. Antibiotics fight off some bugs, disrupting the normal germ colonies.

A few nutritionists advocate yogurt enemas for patients with severe gut infections and yogurt douches for women who get vaginal infections.

Study touts yogurt bacteria for kids

Baltimore Sun

BALTIMORE — Two of the "good bacteria" used in some yogurts can protect children from catching or spreading diarrhea — a common childhood ailment in the United States and a major killer in the Third World, doctors said Thursday.

Pediatric researchers at the Johns Hopkins Children's Center here found that children given a regular diet of infant formula laced with bifida and thermophilus, the live cultures, were 78 percent less likely to get the disease than youngsters who drank plain formula.

Dr. Robert Yolken said the live bacteria are sold as supplements in health food stores but also are present in some cultured milk products including yogurt and acidophilus milk — a product geared for people who cannot digest ordinary milk.

"We might be able to put (the bacteria) into milk delivered to schools and day-care centers," Yolken said.

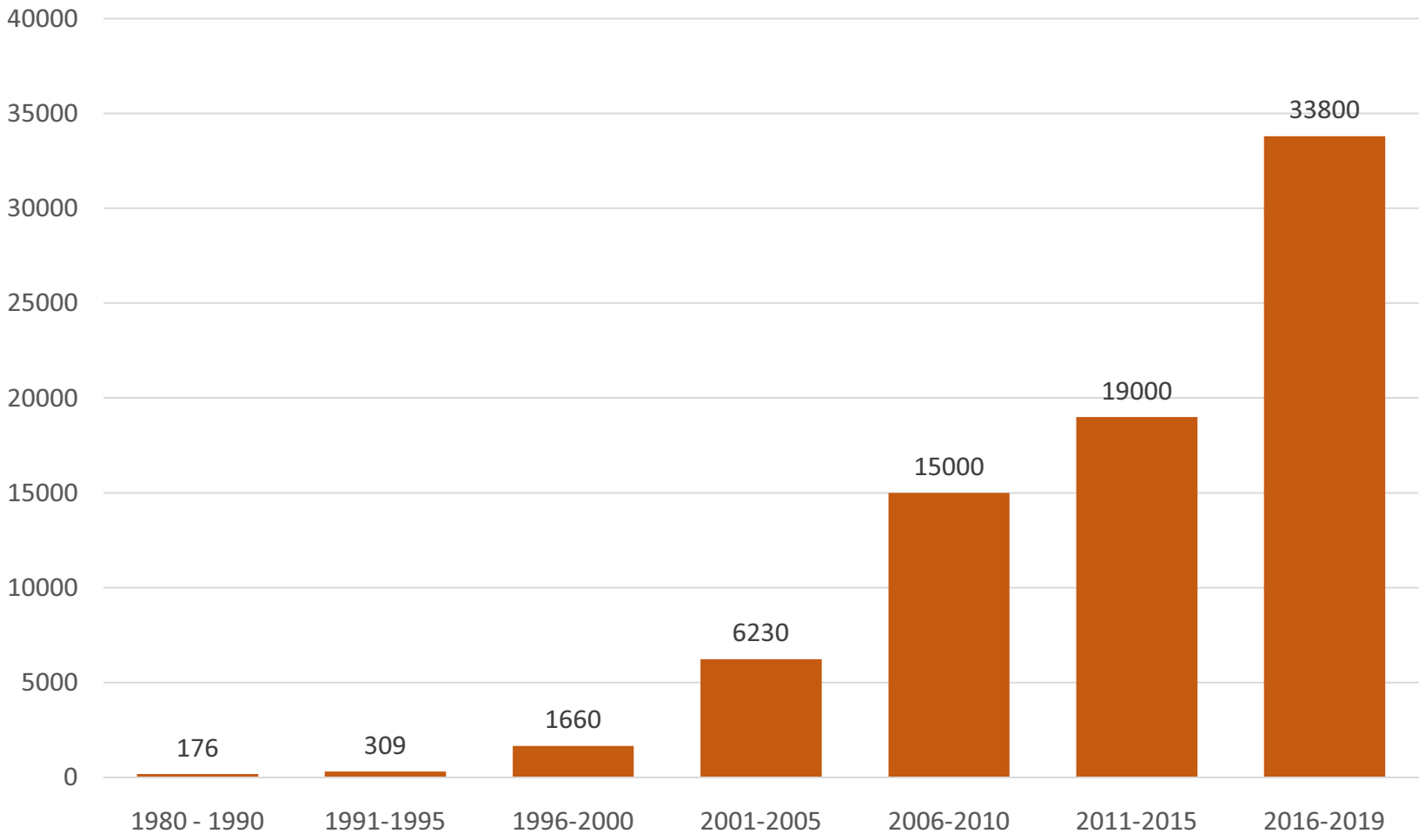
He cautioned, however, that further research is needed to determine who should take it and how much they should take and which bacteria are best.

Nonetheless, it appears that parents can hardly go wrong in feeding yogurt to children who have advanced beyond breast milk or formula. Dr. Jose Saavedra, a pediatric gastroenterologist, said yogurt is a good source of calories, protein and important minerals such as calcium and is easy to digest.

Yolken said yogurt can serve as an excellent bridge between breast milk or formula, which children often give up in the second six months of life, and plain cow's milk, which is difficult for many children to digest.

The study appears in Lancet.

Probiotics: Citations per year

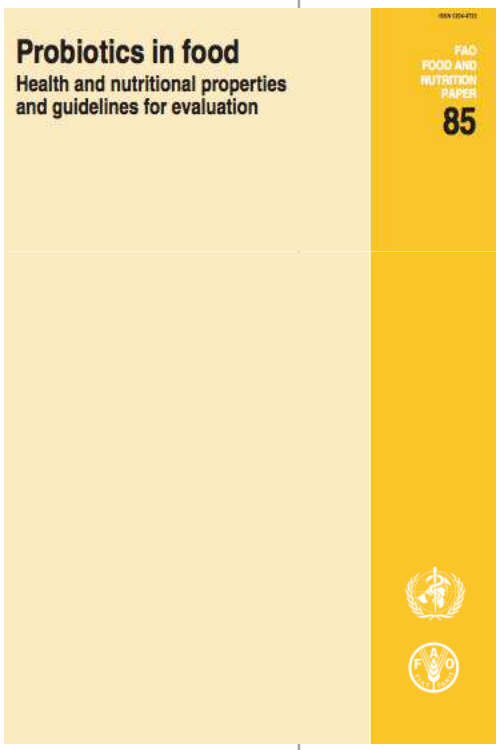


ogle Scholar search 2019: Probiotics, Human medicine

efining obiotics

CONSENSUS STATEMENTS

INTERNATIONAL SCIENTIFIC ASSOCIATION FOR PROBIOTICS AND PREBIOTICS



EXPERT CONSENSUS DOCUMENT

The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic

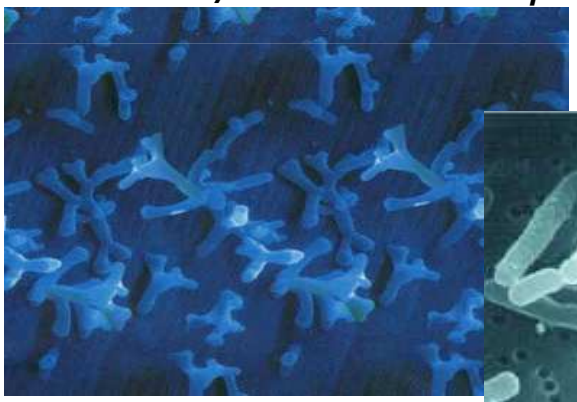
'Live organisms which when administered in adequate amounts confer a health benefit on the host

Hill, C. *et al. Nat. Rev. Gastroenterol. Hepatol.* **11**, 506–514 (2014); published online 10 June 2014; doi:10.1038/nrgastro.2014.66

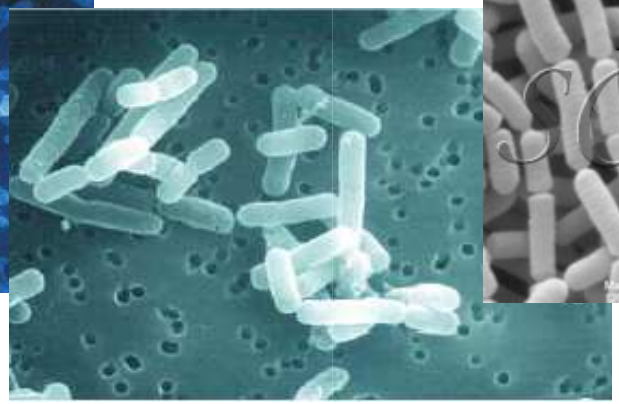
Definition

- *Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host*

FAO/ WHO – adapted by ISAPP



B lactis

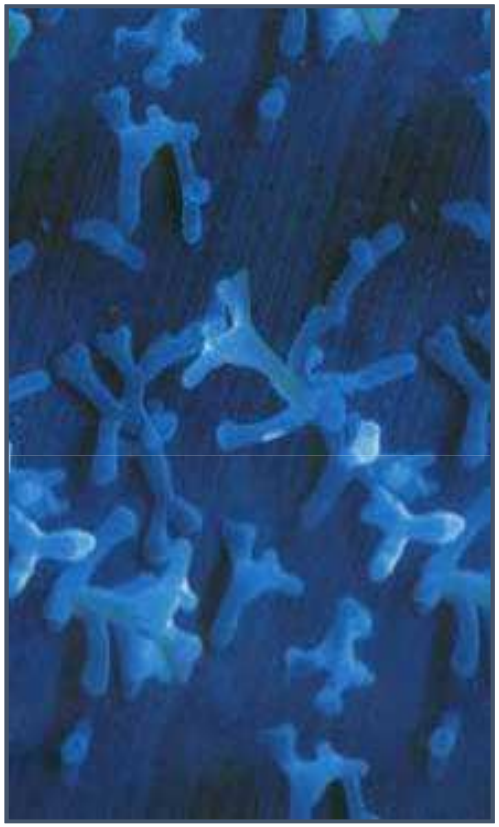


L. reuteri

L. Rhamnosus GG

* *B. lactis* and *L reuteri* are the only two probiotics with GRAS status approved for use in term infant formula from day 1 by US FDA

Bifidobacterium lactis



B.lactis, strain Bb-12

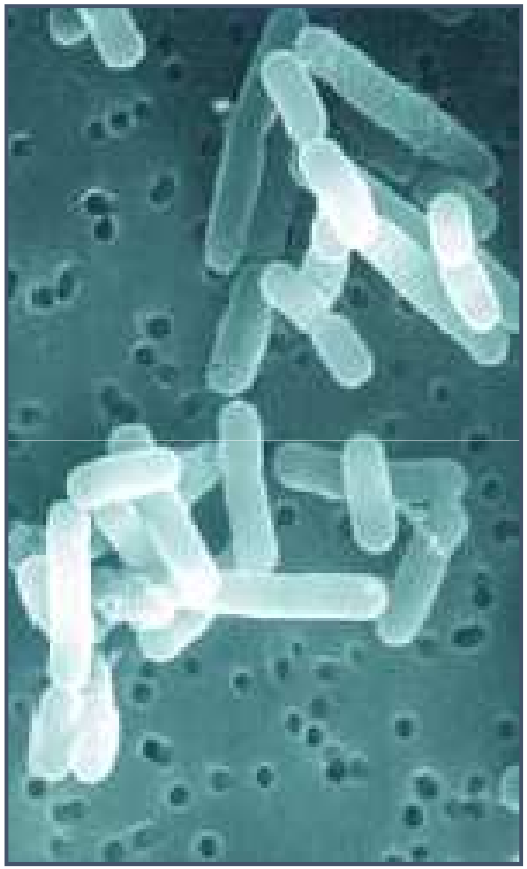
- Bifidobacteria found in breastmilk
  - **Predominant bacteria in the gut of breastfed infants**

Positive outcomes reported:

- Immune system development and modulation
- Increase in Secretory IgA
- Reduce risk of acute diarrhea
- Antibiotic diarrhea risk reduction
- Emerging Evidence in Allergy and NEC
- **FDA GRAS status in infant formula from birth**

enclature - *B. lactis* also: *B. animalis* sub-species *lactis, B. bifidum*

Lactobacillus reuteri



L. reuteri
strain ATCC55730, DSM 17038

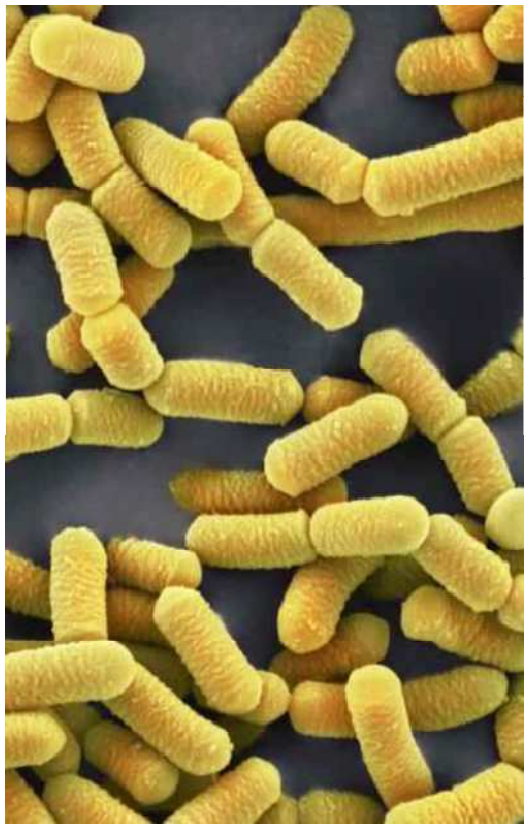
- **Isolated from breastmilk**

Most relevant areas of clinical research showing positive results:

- Reduced infant colic and crying
- Improved GI motility
- Reduced intensity of abdominal pain
- Regulated bowel movements
- Improved feeding tolerance in premature infants
- Diarrhea
- **FDA GRAS status in term infant formula from birrth**

clature – L. reuteri: Parent strain ATCC 55730, cured strain DMS 17938

Lactobacillus rhamnosus (GG)



L. Rhamnosus

- **The most studies probiotic bacterium**

Most relevant areas of clinical research showing positive results:

- Improved GI motility / constipation
- Management of diarrhea
- Diarrhea / acut respiratory infections
- Antibiotic associated diarrhea
- Allergic manifestations
- **No FDA GRAS status in term infant formula from birth. Approved for therapeutic formulas**

ps://www.ingredientsnetwork.com/chr-hansen-lgg-news042798.html

menclature –

GASTROENTEROLOGY 1992;102:875–878

Fecal Recovery in Humans of Viable *Bifidobacterium* sp Ingested in Fermented Milk

YORAM BOUHNIK, PHILIPPE POCHART, PHILIPPE MARTEAU, GUILLAUME ARLET, ISABELLE GODEREL, and JEAN CLAUDE RAMBAUD

Unité de Recherches sur les Fonctions Intestinales, le Métabolisme et la Nutrition, INSERM U290, Hôpital Saint Lazare, Paris; Département de Microbiologie–Immunologie, Unité de Formation et de Recherche des Sciences Pharmaceutiques, Université Paris XI, Châtenay Malabry; and Service de Bactériologie et de Virologie, Hôpital Saint-Louis, Paris, France

***fidobacterium* sp is a natural component of the minant colonic microflora that was recently in-oduced into several fermented dairy products. he aim of the present study was to study the fate of is microorganism in the human gut. On the basis antibiotic resistance characters, a variant of *Bi-obacterium* sp that could be distinguished from digenous bifidobacteria in the fecal flora was se-cted, and its survival and colonization in the co-n was examined. This strain was used to ferment lk, and 125 g of the fermented product obtained as ingested by eight healthy volunteers three nes daily for 8 days. Stools were recovered and eighed throughout the study. The results showed at the exogenous *Bifidobacterium* sp appeared in e stools and reached a mean level of 8.8 ± 0.1 log lony-forming units per gram. This level was aintained as long as the fermented dairy product as consumed. When its ingestion stopped, the ex-enous *Bifidobacterium* sp gradually decreased d was no longer detectable 8 days after cessation. he mean recovered quantity during the 8-day pe-od of administration of the ingested bifidobacteria creted in stools was 12.1 ± 0.1 log colony-forming its per gram, i.e., 29.7% ± 6% of the ingested bac-ria, which was similar to the percentage that ached the colon in previous studies. It is con-uded that under physiological conditions, exoge-usly administered *Bifidobacterium* sp do not lonize the human colon. However, the high fecal ncentrations of exogenous bifidobacteria reached e compatible with metabolic "probiotic" activi-s.**

n many parts of the world, fermented milk consti-tutes a significant and increasing part of food con-mption and usually contains more than 10 billion ing bacteria per 100 g of product. Since Metchni-ff's theories about the beneficial effects of lactic d bacteria on the intestinal microflora,[1] there has en sustained interest in these microorganisms.[2] In-deed, they could fulfil the criteria of probiotic agents, recently redefined as "a live microbial feed supplement which beneficially affects the host animal by improving its microbial balance."[3] In fact, little is known about the fate of ingested bacteria in the organism. After ingestion of a fermented dairy product (FDP), 1.5% of two *Lactobacillus* strains were shown to survive at the terminal ileum[4,5]; also, there was a significant increase in the fecal counts of lactobacilli with another strain.[6] This increase was considered as an explanation for some of the modifications of fecal bacterial enzymatic activities observed after ingestion of living *Lactobacillus* sp in an FDP.[7,8]

However, it is difficult to assess the capacity for survival of an exogenous microorganism in the human colon because of the difficulty of distinguishing exogenous bacteria from their possible endogenous congeners in the fecal flora and sometimes because of the difficulty of clearly identifying certain species among the complex intestinal flora.[9]

If some exogenous bacteria belonging to the subdominant flora do survive in the digestive tract, they cannot colonize the intestinal tract; this led to the concept that the dominant anaerobic component of the indigenous intestinal microflora exerts resistance to colonization by exogenous microorganisms.[10,11] This "barrier effect," the mechanism of which is still obscure, has been shown with several bacteria in which fecal excretion was compared with that of an inert marker ingested at the same time. The barrier effect is considered to be caused by bacteriostasis when the fecal elimination of the two components shows the same kinetics and to bacteriolysis when the exogenous bacteria is eliminated quicker than the passive marker.[12] However, there has been no direct experimental proof in either human subjects or experimental animals whether this barrier effect also applies to species belonging to the dominant flora.

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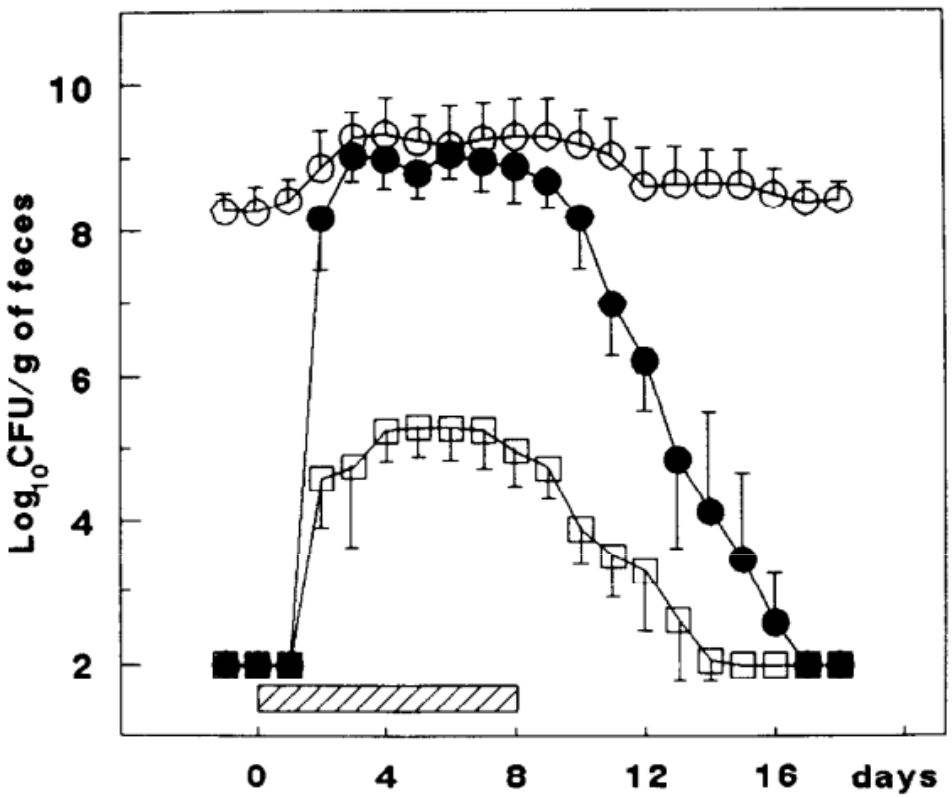


Figure 1. Fecal concentrations (mean ± SE) of total bifidobacteria (○), a selected strain of *Bifidobacterium* sp (BOSR) (●), and spores of *Bacillus stearothermophilus* (SBS) (□) obtained in eight healthy volunteers after ingestion of 125 g t.i.d. of fermented dairy product (▨) containing 9.2 log CFU/g of BOSR and 5.4 log CFU/g of SBS for 8 days.

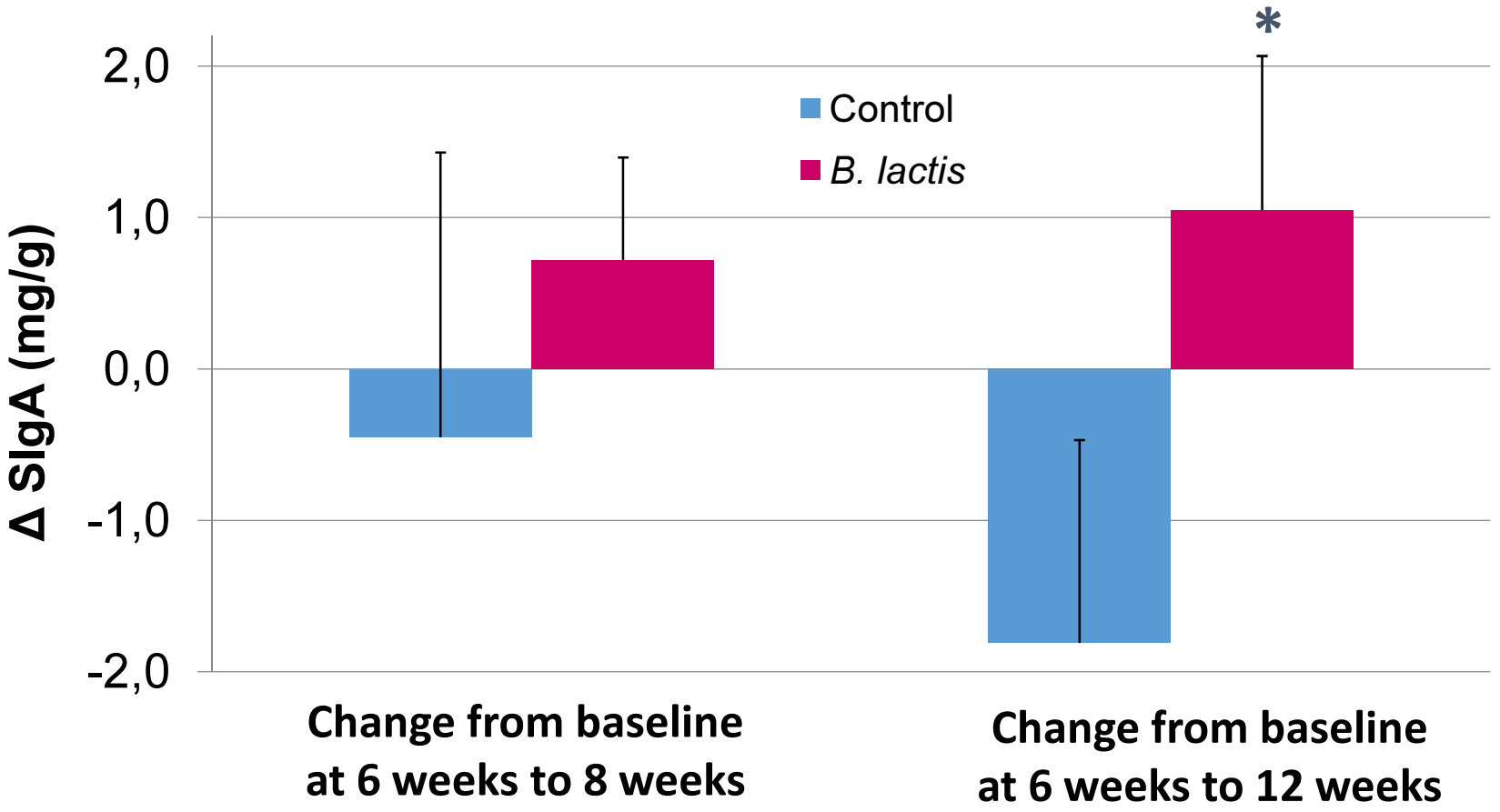
nik Y et al Gastroenterology, 1992

Documented Effects of Probiotic Supplementation on Protective Gut Barrier and Immune Function

- Decreased gut permeability
- Increased mucin production
- Increased IgA secreting cells and secretory IgA
- Increased natural killer cell tumor-killing activity
- Increased production of macrophages and activated phagocytosis
- Immune modulation towards antigen tolerance

Fukushima Y., et al. Int J Food Micorbiol 1998;42:39-44
Rautava S., et al. Pediatr Res 2006;60:221-224
Stratiki Z., et al. Early Hum Dev 2007 on-line at: http://dx.doi.org/10.1016/j.earlhumdev.2006.12.002

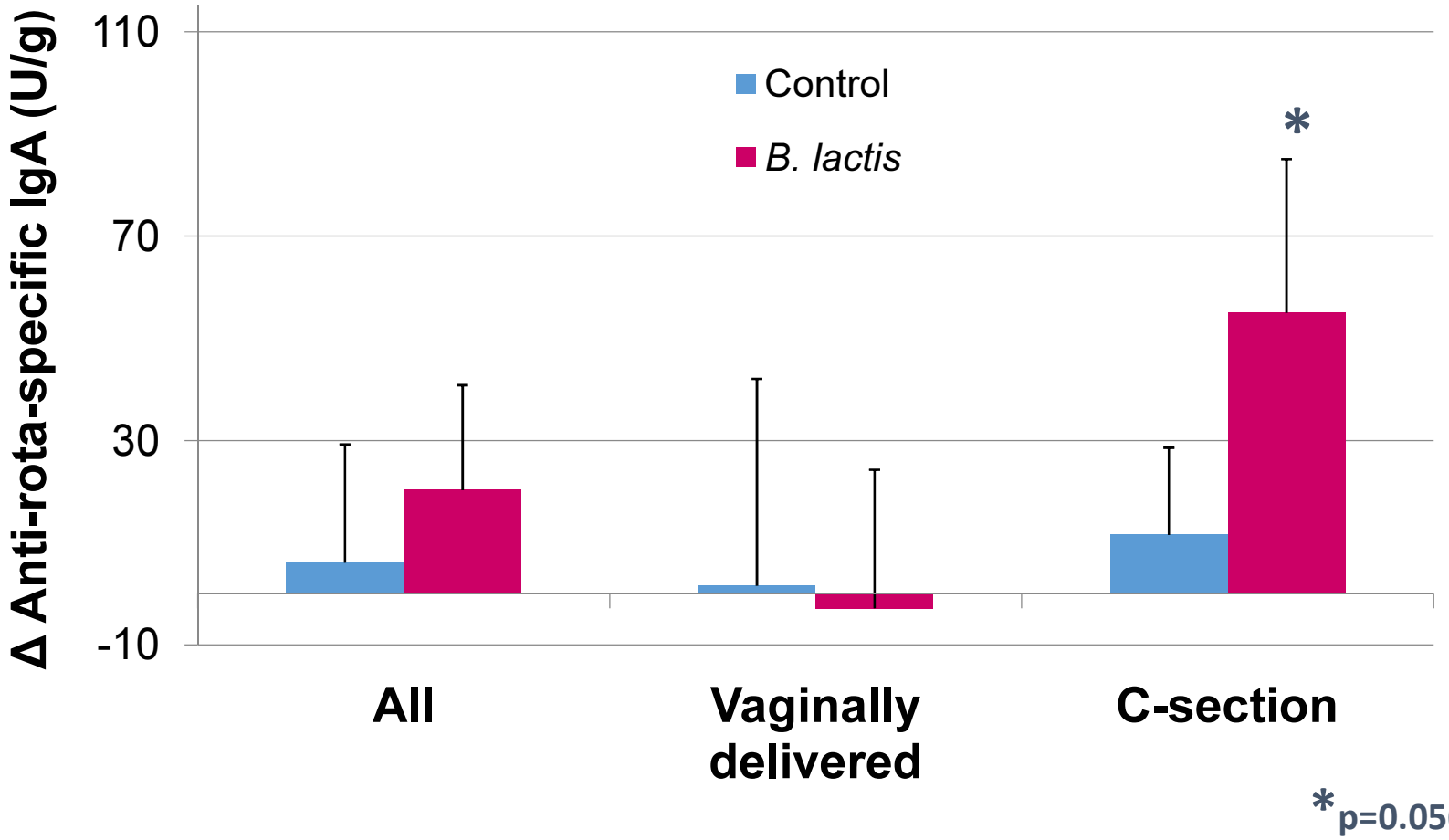
Infant Formula Supplemented with B. lactis Increases Fecal IgA



Holscher H. J Parenter Enteral Nutr. 2012;36:106S-17S 2012.

*p<0.05

Anti-Rotavirus IgA Increases with *B. lactis* Supplementation in Infants Born via C-section



Holscher H. J Parenter Enteral Nutr. 2012;36:106S-17S 2012.

Clinical Outcomes reported with specific probiotics documented in Infants and Children

- Modification of intestinal microbiota
- Reduced risk and duration of acute diarrhea
- Reduced risk of respiratory infections
- Reduce crying time in Infant colic
- Reduced risk of atopic dermatitis
- Reduced risk of NEC

Langhendries JP, et al. J Pediatr Gastroenterol Nutr. 1995; 21(2):177-181
Agostoni C, et al. J Pediatr Gastroenterol Nutr. 2004:38:365-374

Prevention of nosocomial diarrhoea

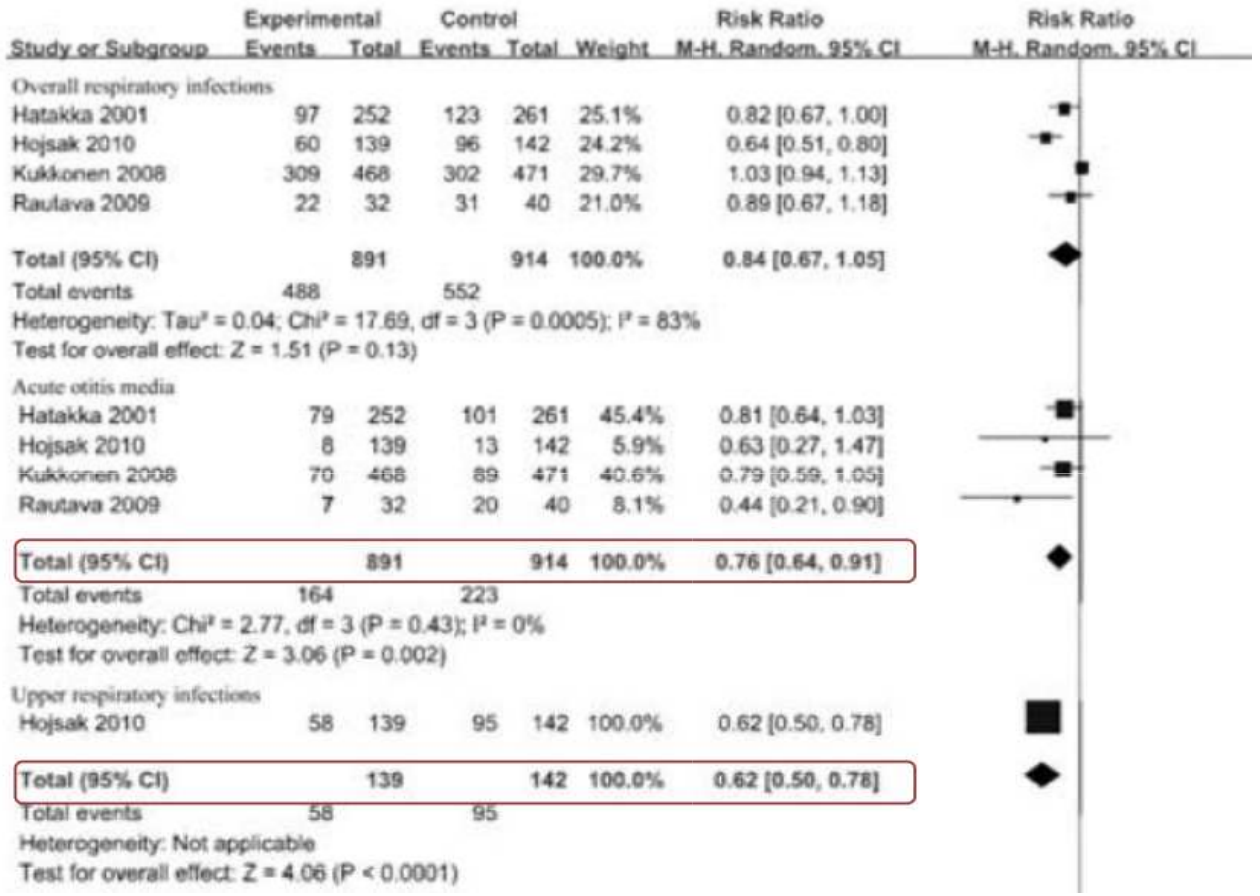
		RR (95% CI)	NNT (95% CI)
Saavedra Lancet 1994	*B. Bifidum* *+ S. therm*	0.2 (0.06-0.8)	5 (3-20)
Penna Pediatria 2009	*L. delbrueckii* *H2B20*	1.6 (0.6-4.0)	NS
Szajewska J Pediatr 2000	*L. rhamnosus GG*	0.2 (0.06-0.6)	4 (2-10)
Mastretta JPGN 2002	*L. rhamnosus GG*	0.8 (0.6-1.3)	NS
Hojsak Pediatrics 2010	*L. Rhamnosus GG*	0.4 (0.25-0.7)	15 (9-34)

Probiotics in preventing infections in day care centers

	Probiotic	Diarrhea	Respiratory inf.
Ribeiro 1998	*L plantarum 299v*	**Decreased**	**Decreased**
Pedone 2000	*L casei DN 114 001*	**Decreased**	**NA**
Chouraqui 2004	*B lactis Bb12*	**Decreased**	**NA**
Hatakka 2001	*LGG*	**NS**	**Decreased**
Thibault 2004	*B breve + Str therm*	**Decreased**	**NA**
Saavedra 2004	*B lactis + Str therm*	**NS**	**NS**
Weizman 2005	*B lactis OR L reuteri ATCC 55730*	**Decreased**	**NS**
Binns 2007	*B lactis + FOS + GUM*	**Decreased**	**NS**
Waligora 2006	*Oligofructose*	**Decreased**	**NS**
Leyer 2009	*L acidophilus + B animalis*	**N/A**	**Decreased**
Merenstein 2010	*L casei DN 114 001*	**Decreased**	**Decreased**
Hojsak 2010	*L rhamnosus GG*	**NS**	**Decreased**
Agustina 2012	*L reuteri DSM 17938*	**Decreased**	**NS**
Gutierrez-Castrellon 2014	*L reuteri DSM 17938*	**Decreased**	**Decreased**

Weizman Z. Beneficial Microbes 2014 doi 10.3920/BM2014.0101

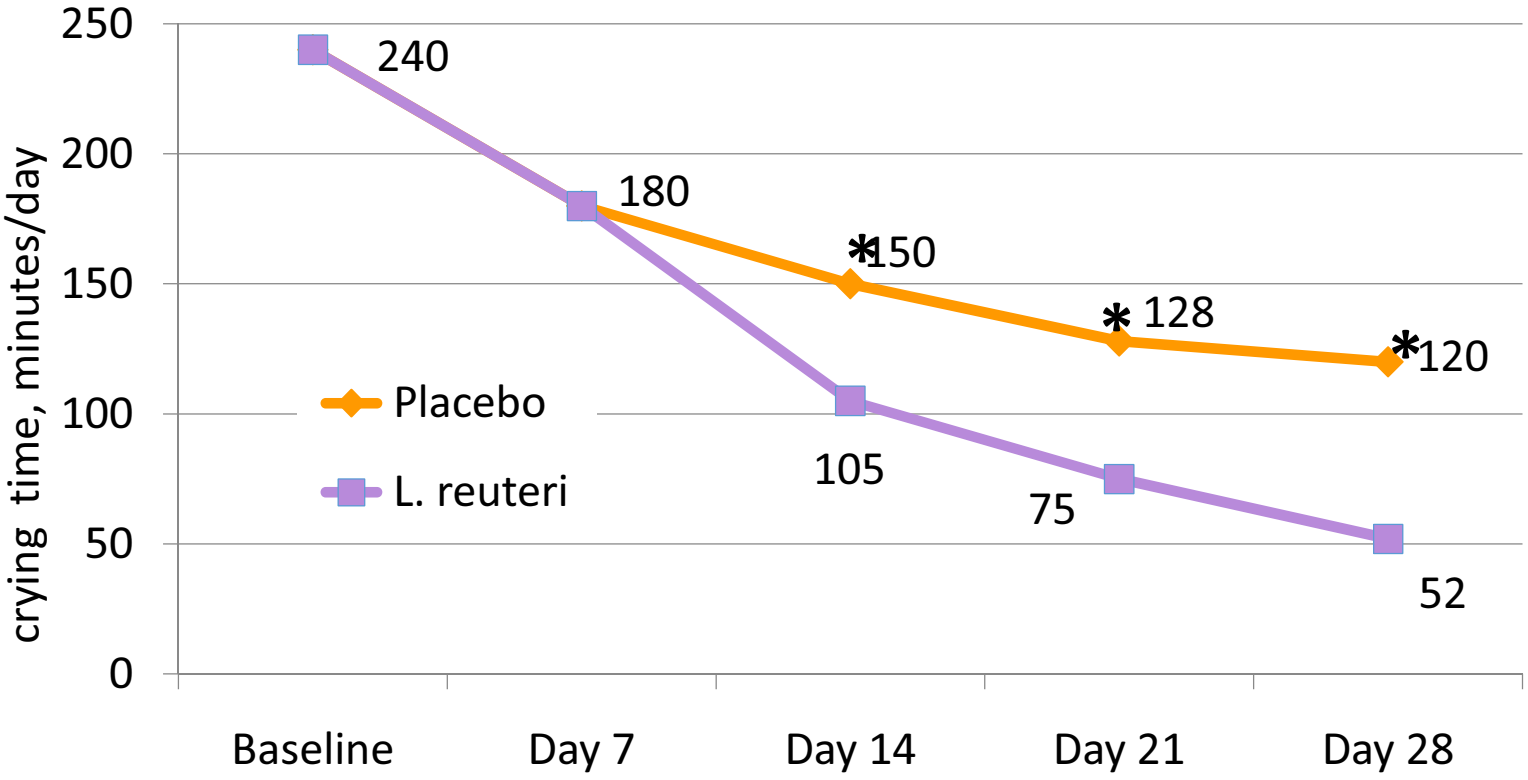
L. rhamnosus GG and respiratory infections



Overall respiratory infections *2 RCTs, n=794, RR* 0.73, 95% CI:0.57-0.92).
Otitis media *4 RCTs, n=1805, RR* 0.76, 95% CI: 0.64-0.91)
Upper respiratory infections 1 RCT, n=281, RR 0.62, 95% CI 0.50-0.78)

Liu 2013

Reduction in Crying Time with *L. reuteri* Supplementation vs. Placebo



*p<0.0001

Szajewska, H., et al. *J Pediatr* 2013;162(2):257-62.

Potential Mechanisms by which Probiotics May be Beneficial in Infant Colic

- Promote microbial balance (increase lactobacilli & decrease coliforms and bacteroides)[1]
- Improve gut motility in infants[2]
- Enhance mucosal barrier (decrease gut permeability)[3]
- Neuro - chemical mechanism through the gut-brain axis (?)

1. Savino, F, et al. Pediatrics 2010; 126(3):e526-33.
2. Indiro F, et al. J Peds 2008;152(6):801-6.
3. Savino F, et al. Pediatrics 2007;119(1):e124-30.
4. Rosenfeldt V. et al. J Pediatr 2004:145:612-16.

Probiotics in Prematures Effect on Stage 2 NEC

	Probiotic (n/N)	No probiotic (n/N)	RR (fixed) (95% CI)	Weight (%)	RR (95% CI)
Kitajima, 1997[26]	0/45	0/46			Not estimable
Dani, 2002[20]	4/295	8/290		20·76	0·49 (0·15–1·61)
Costalos, 2003[23]	5/51	6/36		18·10	0·59 (0·19–1·78)
Bin Nun, 2005[22]	1/72	10/73		25·55	0·10 (0·01–0·77)
Lin, 2005[21]	2/180	10/187		25·23	0·21 (0·05–0·94)
Manzoni, 2006[24]	1/39	3/41		7·52	0·35 (0·04–3·23)
Mohan, 2006[25]	2/21	1/17		2·84	1·62 (0·16–16·37)
Total (95% CI)	703	690		100·00	0·36 (0·20–0·65)

Total events: 15 (probiotic), 38 (no probiotic)
Test for heterogeneity: χ^2=4·66, df=5 (p=0·46), I^2=0%
Test for overall effect: Z=3·37 (p=0·0008)

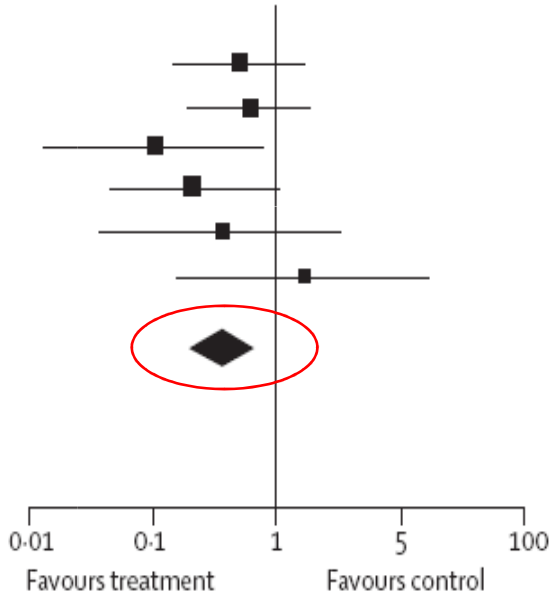


Figure 2: Effect of probiotics on necrotising enterocolitis of stage 2 or greater

73% Risk reduction

Probiotics in Prematures: Mortality

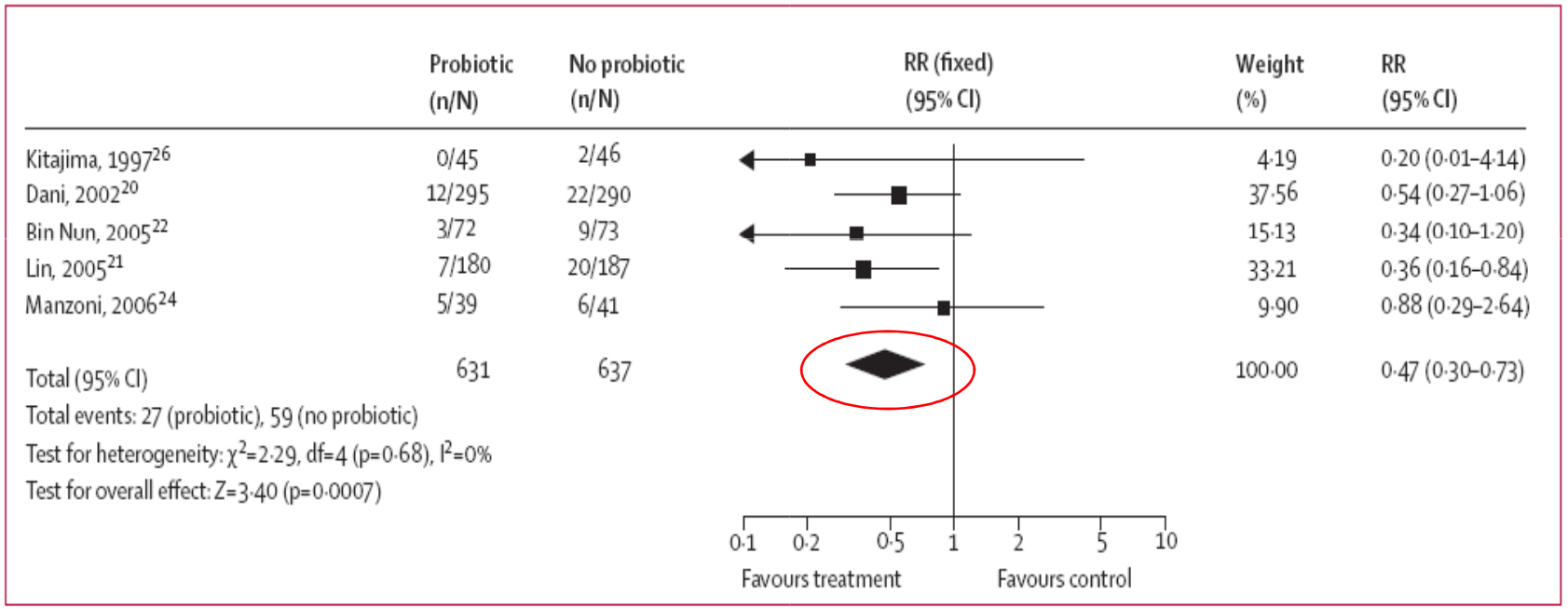


Figure 4: Effect of probiotics on all-cause mortality

53% Risk reduction

Brief general summary: Clinical use of probiotics*

The use of *specific probiotics* can

- Decrease risk and duration of acute diarrhea in healthy infants

  B.lactis, B. bifidum, B. infantis, LGG, L. reuteri, S boulardi
- Decrease in antibiotic assoiated diarrhea

  L.GG, [S. boulardi]
- Decrease respiratory infections

  L.GG, L. reuteri
- Decrease crying time in infants with colic

  L. reuteri
- Decrease NEC in premature infants

  Bifidobacteria & lactobacilli
- Decrease allergic manifestations

  L. GG

t exhaustive or all encompassing

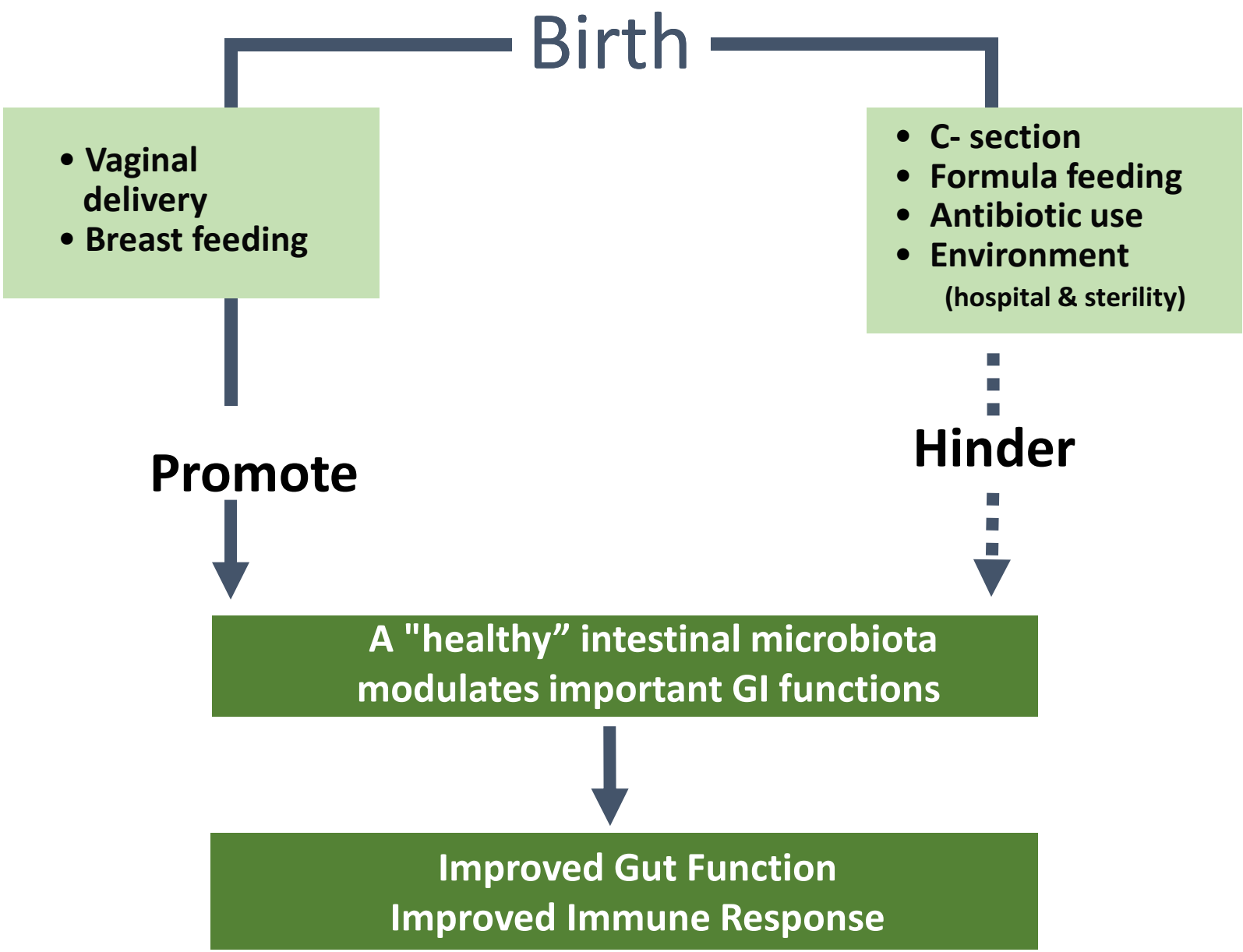
Considerations when interpreting probiotic studies

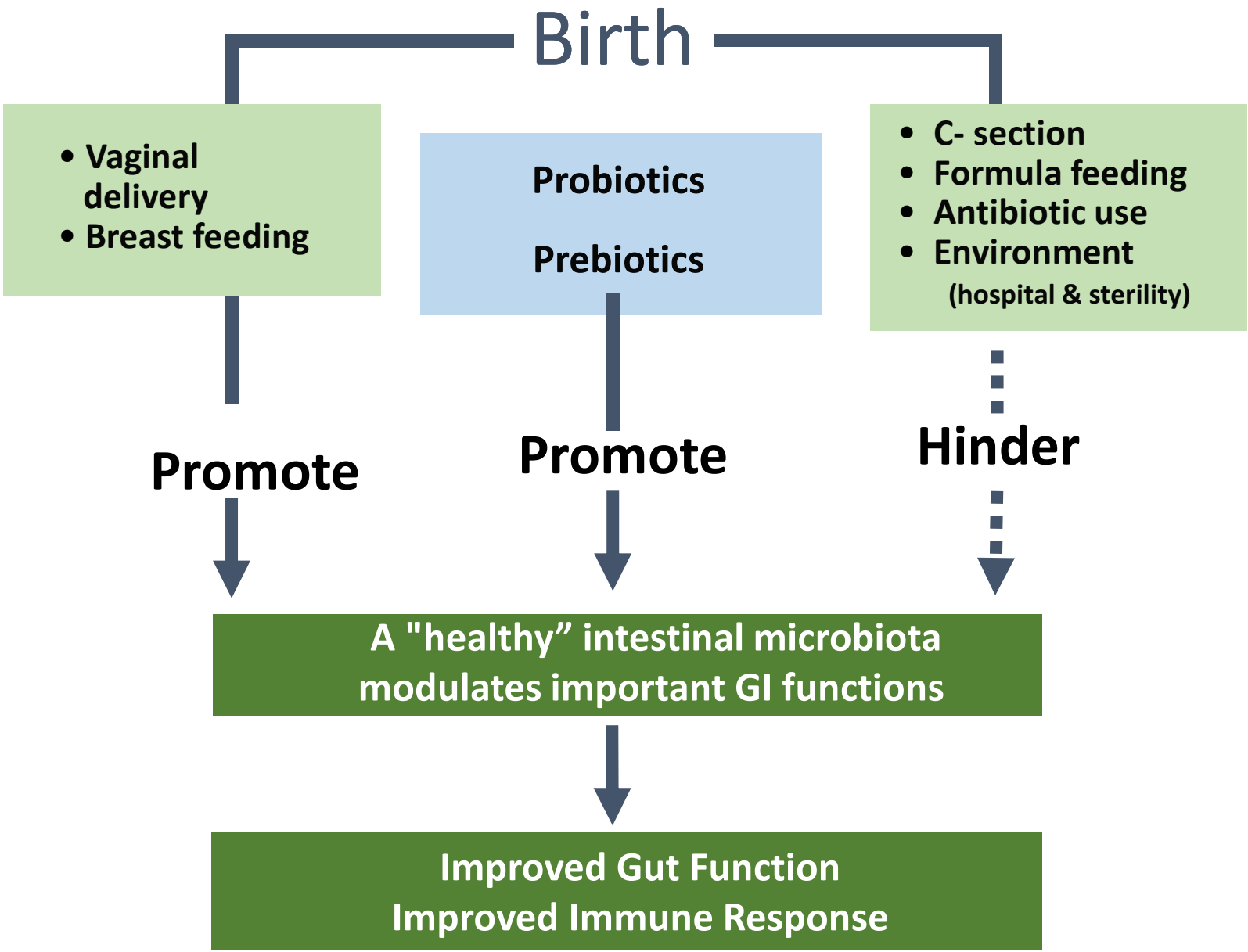
Not all probiotics are created equal

- Consider genus, species and strain
- Dose
- Mode of administration

Not all hosts are created equal

- Different risk populations
- C section
- Breastfeeding
- Diet
- Antibiotics


Birth
• Vaginal delivery
• Breast feeding
• C- section
• Formula feeding
• Antibiotic use
• Environment
(hospital & sterility)
Promote
Hinder
A "healthy" intestinal microbiota modulates important GI functions
Improved Gut Function
Improved Immune Response


Birth
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• Formula feeding
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Thank You

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Next-Generation Sequencing Challenges

GS Growing By Leaps and Bounds, Problems Arise

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