



# **INTESTINAL MICROBIOLOGY IN EARLY LIFE**

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**ISAPP, 5<sup>th</sup> June 2018, Singapore**

# THE GUT MICROBIOTA

**100 million  
Neurons**

**Surface of 250m<sup>2</sup>**

- Most of our “outside” is tucked away inside

**100 trillion  
Bacteria**  
(~1,5 kg)

**60 -70%  
Immune Cells**

- Digestion
- Adsorption
- Barrier function
- Innate defense
- Mucus production
- Hormone secretion

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# PATTERNS IN EARLY LIFE MICROBIOTA DEVELOPMENT

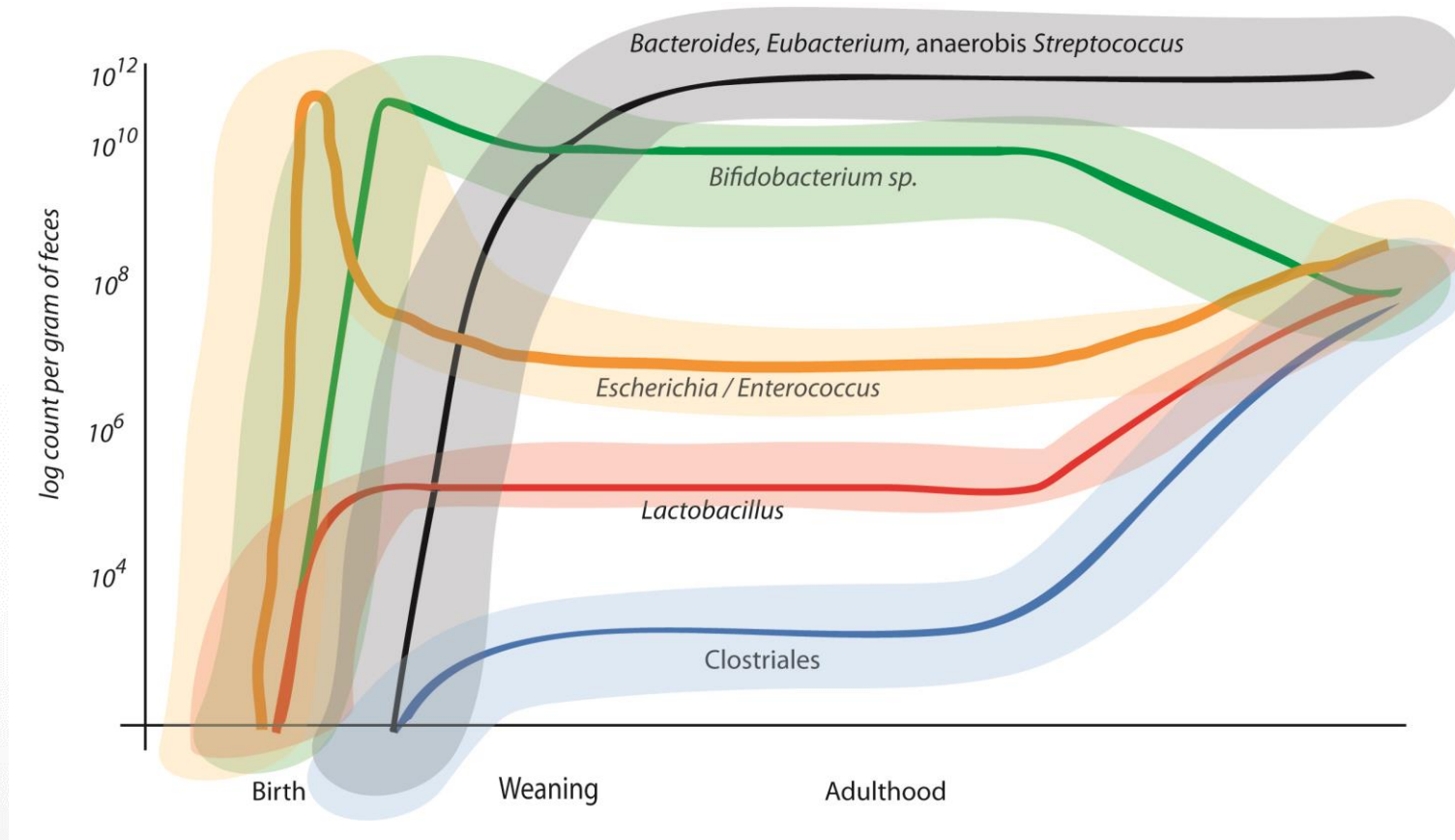


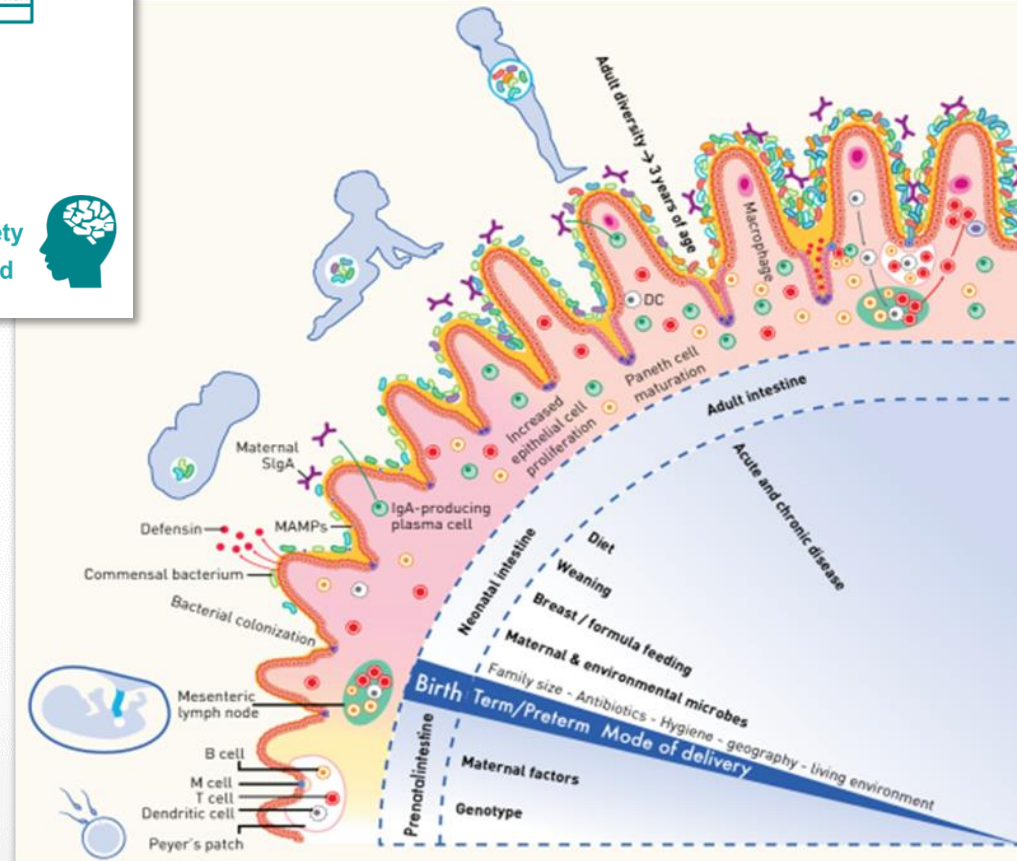
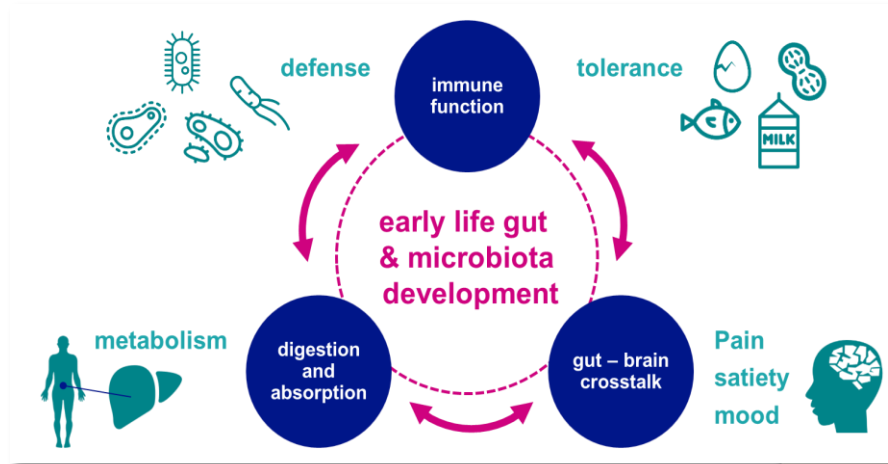
Figure based on Mitsuoka & Hayakawa (1973)

Die Zusammensetzung der Faekalflora der verschiedenen Altersgruppen.

Zbl Bakt Hyg I Orig 233: 333–342.

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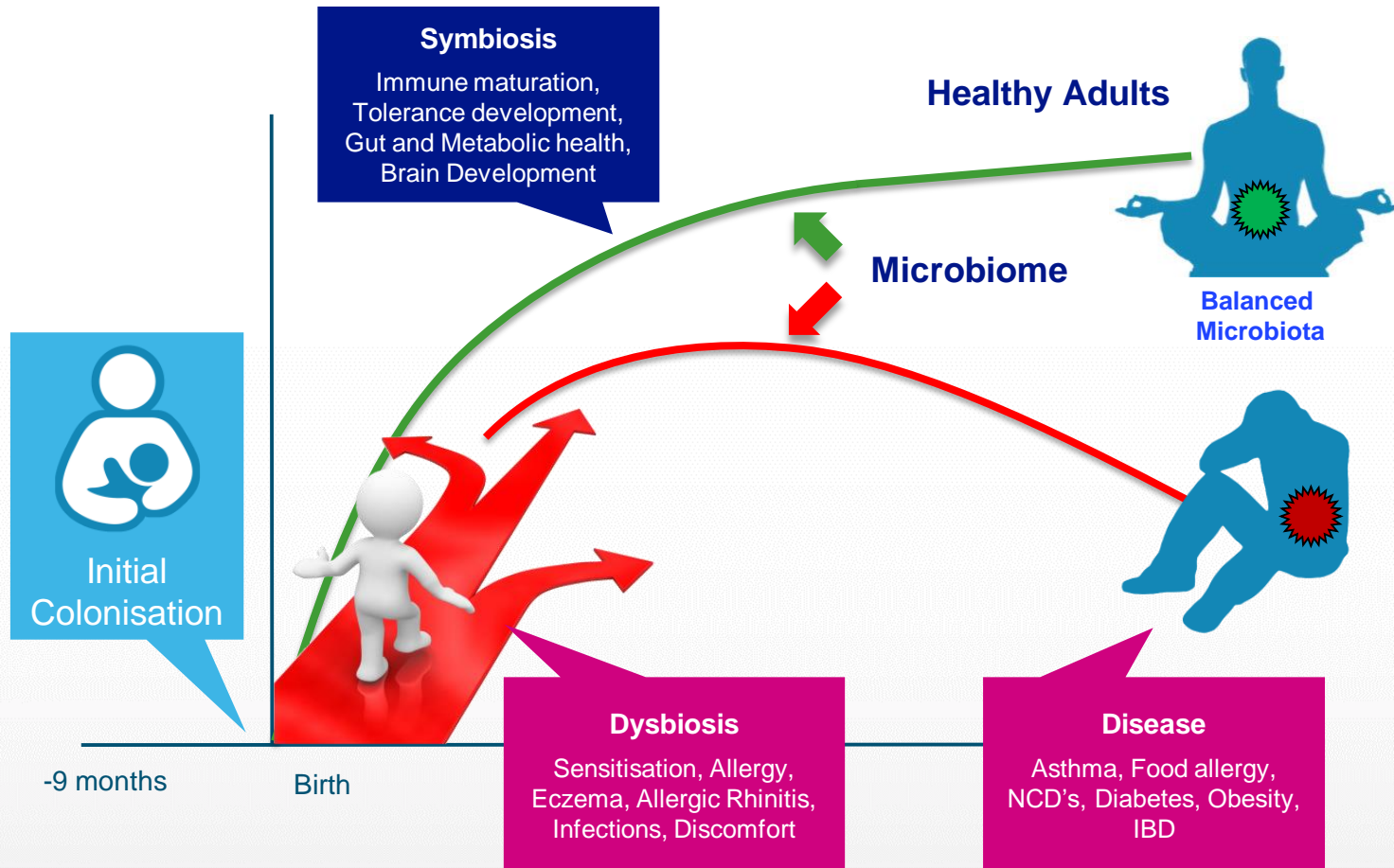
# ESTABLISHING THE SYMBIOSIS



Wopereis H, et al. *Pediatr Allergy Immunol* 2014;25:428-438

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# MICROBIOTA IN EARLY LIFE POWERFUL MEANS FOR IMPROVING OUR HEALTH



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# EARLY MICROBIOME ALTERATIONS & DISEASE RISK

## Infant gut microbiota composition could be early allergy indicator: Danone

By Cheryl Tay <sup>1</sup>

20-Oct-2017 | Last updated on 20-Oct-2017 at 03:06 GMT



Elevated pathogen / bacteria ratio in infants could be an early microbiota biomarker for allergies, according to researchers in Singapore, the UK and the Netherlands.

Korpela et al. *Microbiome* (2017) 5:26  
DOI 10.1186/s40168-017-0245-y

Microbiome

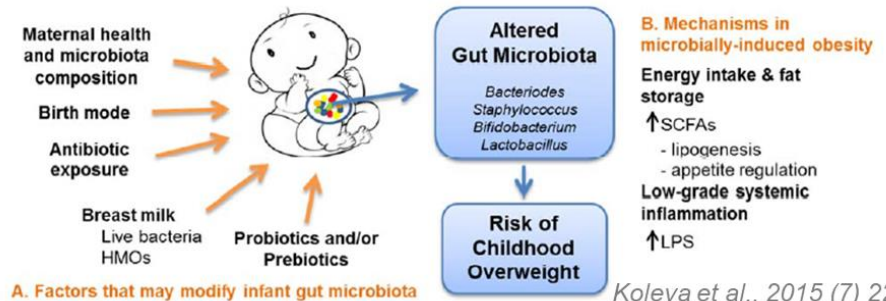
RESEARCH

Open Access



## Childhood BMI in relation to microbiota in infancy and lifetime antibiotic use

K. Korpela<sup>1\*</sup>, M. A. C. Zijlman<sup>2</sup>, M. Kuitunen<sup>3</sup>, K. Kukkonen<sup>4</sup>, E. Savilahti<sup>3</sup>, A. Salonen<sup>1</sup>, C. de Weerth<sup>2</sup> and W. M. de Vos<sup>1,5</sup>



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# MICROBIOME DIFFERENCES PRECEDE THE ONSET

Beneficial Microbes, 2017 online

ARTICLE IN PRESS



Ratio of *Klebsiella/Bifidobacterium* in early life correlates with later development of paediatric allergy

J.S.Y. Low<sup>1</sup>, S.-E. Soh<sup>2,3#</sup>, Y.K. Lee<sup>4</sup>, K.Y.C. Kwek<sup>5#</sup>, J.D. Holbrook<sup>2,6</sup>, E.M. Van der Beek<sup>7,8</sup>, L.P. Shek<sup>2,3#</sup>, A.E.N. Goh<sup>5#</sup>, O.H. Teoh<sup>5#</sup>, K.M. Godfrey<sup>9#</sup>, Y.-S. Chong<sup>2,10#</sup>, J. Knol<sup>7,11</sup> and C. Lay<sup>1,3\*</sup>

## Infant gut microbiota composition could be early allergy indicator: Danone

By Cheryl Tay



Elevated pathogen / bacteria ratio in infants could be an early microbiota biomarker for allergies, according to researchers in Singapore, the UK and the Netherlands.

Table S2. Ratio of relative abundances of *Klebsiella* to *Bifidobacterium* (K/B ratio) of allergic and healthy infants at age 3 weeks, 3 months and 6 months respectively.

Timepoint	<i>Klebsiella/Bifidobacterium</i> ratio		
	Healthy controls	Allergic cases	P-value
Week 3	0.23 (0.01-213.07)	1.70 (0.01-1408.00)	0.12
Month 3	0.03 (0.00-0.68)	0.52 (0.00 – 2281.33)	*0.01
Month 6	0.01 (0.00-62.00)	0.05 (0.00-910.50)	0.26

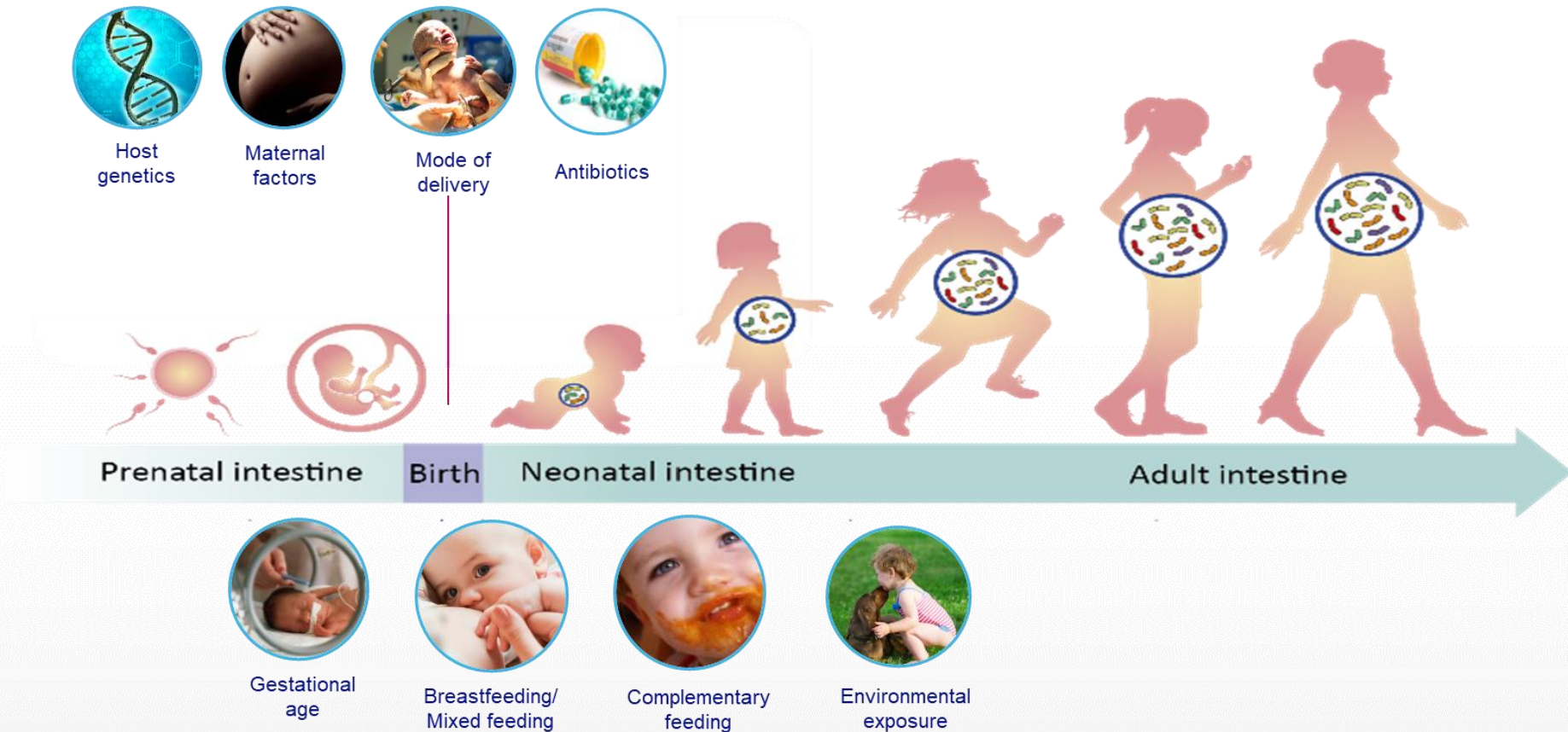
Differences precede the onset

The values were expressed in median (range). Differences in medians of *Klebsiella/Bifidobacterium* ratio between the two groups were performed using non-parametric Mann-Whitney U test. Single asterisk (\*) indicate  $P \leq 0.05$ .

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# FACTORS IMPACTING EARLY COLONIZATION



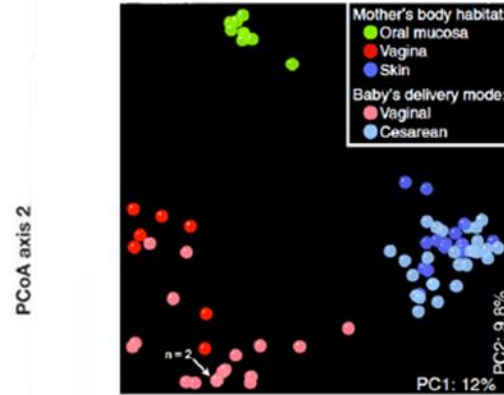
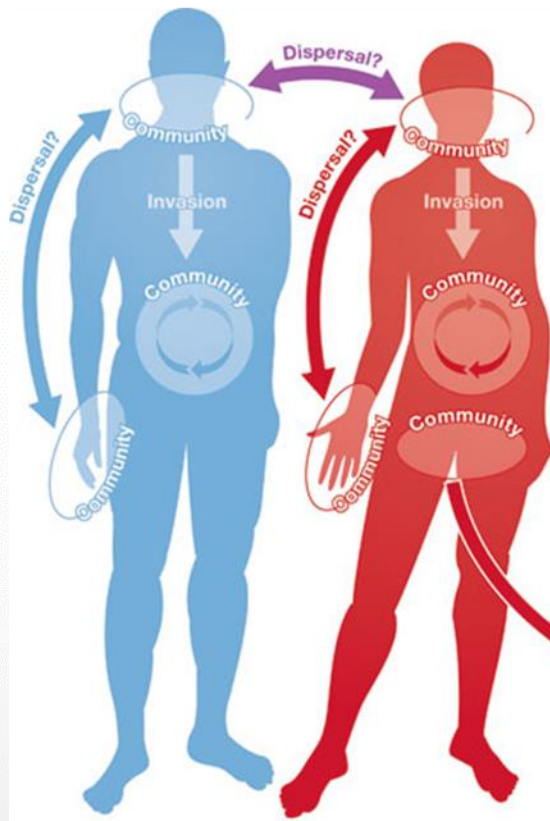
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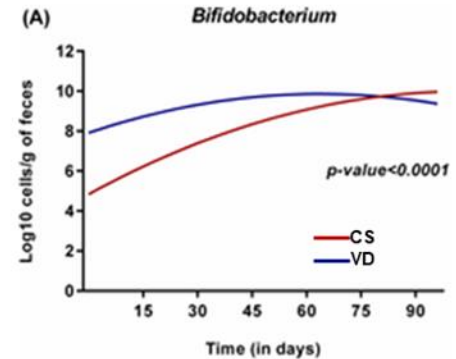
# ACQUISITION OF THE MICROBIOME IN EARLY LIFE BY VERTICAL TRANSMISSION



Infants born by C-section have a delayed colonization



Dominguez-Bello et al., 2010, PNAS 107:11971-11975



Martin et al., 2016, PlosONE 11 (6)

1000 DAYS

# ASSOCIATIONS BETWEEN FACTORS IMPACTING EARLY COLONIZATION AND HEALTH OUTCOMES



<http://www.dailymail.co.uk/home/index.html>

## Babies delivered by Caesarean section at higher risk of asthma and allergies

C. Roduit et al., 2009, *Thorax*, 64(2):107-13

Asthma at 8 years of age in children born by caesarean section

<http://www.healio.com/dermatology/dermatitis/news/>

## Cesarean delivery showed link with gut microbiota, atopic dermatitis

News - Science

Children born by c-section far more likely to be obese by aged five, major study suggests

**Citation:** Keag OE, Norman JE, Stock SJ (2018) Long-term risks and benefits associated with cesarean delivery for mother, baby, and subsequent pregnancies: Systematic review and meta-analysis. *PLoS Med* 15(1): e1002494. <https://doi.org/10.1371/journal.pmed.1002494>

JAMA Pediatrics | Original Investigation

## Association Between Use of Acid-Suppressive Medications and Antibiotics During Infancy and Allergic Diseases in Early Childhood

Edward Mitre, MD; Apryl Susi, MS; Laura E. Kropp, MPH; David J. Schwartz, MD; Gregory H. Gorman, MD; Cade M. Nyland, MD

*Clin Exp Allergy*. 2017 February ; 47(2): 236–244. doi:10.1111/cea.12807.

JAMA Pediatr. doi:10.1001/jamapediatrics.2018.0315  
Published online April 2, 2018.

## Early Life Antibiotic Use and Subsequent Diagnosis of Food Allergy and Allergic Diseases

Annemarie G. Hirsch, PhD, MPH<sup>1</sup>, Jonathan Pollak, MPP<sup>2,3</sup>, Thomas A. Glass, PhD<sup>4</sup>, Melissa N. Poulsen, PhD<sup>1,2</sup>, Lisa Bailey-Davis, DEd, RD<sup>1</sup>, Jacob Mowery, BS<sup>1</sup>, and Brian S. Schwartz, MD, MS<sup>1,2,4</sup>

*Ann Allergy Asthma Immunol* 119 (2017) 54–58

## Influence of antibiotic use in early childhood on asthma and allergic diseases at age 5

Kiwako Yamamoto-Hanada, MD, PhD\*; Limin Yang, MD, PhD\*; Masami Narita, MD, PhD\*; Hirohisa Saito, MD, PhD<sup>†</sup>; Yukihiko Ohya, MD, PhD\*

*Obesity (Silver Spring)*. 2017 February ; 25(2): 438–444. doi:10.1002/oby.21719.

## Associations of Prenatal and Childhood Antibiotic Use with Child Body Mass Index at Age Three Years

Melissa N. Poulsen, PhD, MPH<sup>1,2</sup>, Jonathan Pollak, MPP<sup>1</sup>, Lisa Bailey-Davis, DEd, RD<sup>2</sup>, Annemarie G. Hirsch, PhD, MPH<sup>2</sup>, Thomas A. Glass, PhD<sup>3</sup>, and Brian S. Schwartz, MD<sup>1,2,3,4</sup>

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NUTRICIA RESEARCH



# COMPROMISING THE MATERNAL MICROBIOTA DURING PREGNANCY HAS LONG TERM HEALTH CONSEQUENCES ON THE OFFSPRING

Clin Exp Allergy. 2014 Jun 18. doi: 10.1111/cea.12356. [Epub ahead of print]

## Prenatal and Postnatal Exposure to Antibiotics and Risk of Asthma in Childhood.

Metsälä J<sup>1</sup>, Lundqvist A, Virta LJ, Kaila M, Gissler M, Virtanen SM.

## Maternal antibiotic use and childhood asthma: the missing link?

Lancet Respir Med 2014

Published Online

July 25, 2014

[http://dx.doi.org/10.1016/](http://dx.doi.org/10.1016/S2213-2600(14)70122-5)

[S2213-2600\(14\)70122-5](http://dx.doi.org/10.1016/S2213-2600(14)70122-5)

www.jpeds.com • THE JOURNAL OF PEDIATRICS

## Use of Antibiotics during Pregnancy Increases the Risk of Asthma in Early Childhood

Lone Graff Stensballe, MD, PhD<sup>1,2</sup>, Jacob Simonsen, MSc, PhD<sup>2</sup>, Signe M. Jensen, MSc<sup>1</sup>, Klaus Bønnelykke, MD, PhD<sup>1</sup>, and Hans Bisgaard, MD, DMSc<sup>1</sup>

Lancet Respir Med. 2014 Aug;2(8):631-7. doi: 10.1016/S2213-2600(14)70152-3. Epub 2014 Jul 24.

## Maternal propensity for infections and risk of childhood asthma: a registry-based cohort study.

Stokholm J<sup>1</sup>, Sevelsted A<sup>2</sup>, Bønnelykke K<sup>2</sup>, Bisgaard H<sup>3</sup>.

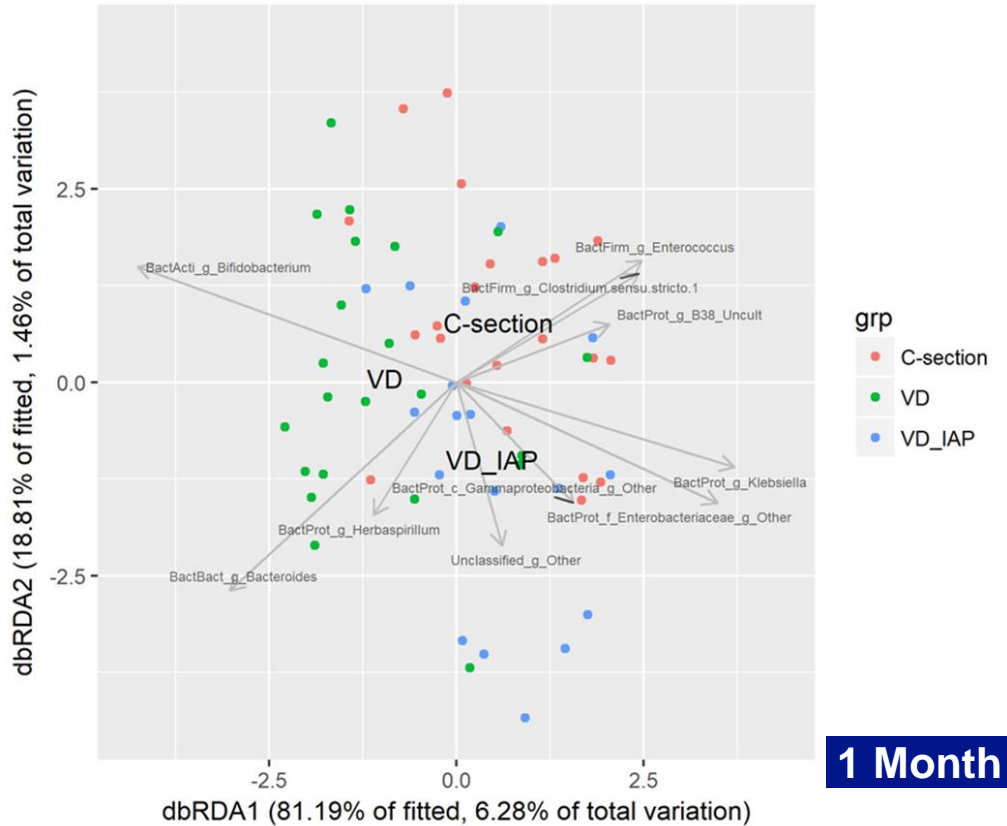
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NUTRICIA RESEARCH

# INTRAPARTUM ANTIBIOTIC PROPHYLAXIS AND C-SECTION DISRUPT THE EARLY COLONIZATION PROCESS

Poster 7833



time point	VD vs VD_IAP	VD vs CS	CS vs VD_IAP
Day 0	0.561	0.023	0.044
Day 7	0.014	0.001	0.034
1 Month	0.006	0.003	0.550
3 Month	0.047	0.002	0.193

## COMPROMISED GUT MICROBIOTA AT BIRTH

Wei Wei Thwe Khine<sup>1,2</sup>, Christophe Lay<sup>3,4</sup>, Mahesh Choolan<sup>5</sup>, Claudia Chi<sup>6</sup>, Jan Kno<sup>7</sup>, Seppo Salminen<sup>8</sup>, Lee Yuen Kun<sup>9</sup>

<sup>1</sup>Department of Microbiology and Immunology, National University of Singapore, <sup>2</sup>School of Medicine, <sup>3</sup>Food Development, <sup>4</sup>Industrial Food, University of Lille, France, <sup>5</sup>Science Nutrition Research, Singapore, <sup>6</sup>Department of Paediatrics, <sup>7</sup>National University of Singapore, <sup>8</sup>Department of Obstetrics and Gynaecology, National University Hospital, Singapore, <sup>9</sup>Canberra National Research, University, Wageningen, The Netherlands

### BACKGROUND AND RATIONALE

The maturation of the gut microbiota in early life is tightly linked with the development of the immune and metabolic systems. Several studies have indicated that a compromised microbiota both and in the first 100 days of life is a risk factor for allergy and obesity.

### OBJECTIVE

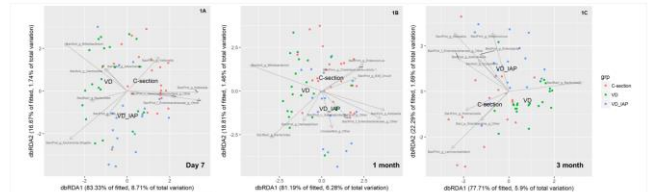
The objective of this study was to determine the effect of mode of delivery and its associated clinical care practice (Vaginal birth, Vaginal birth with intrapartum antibiotic prophylaxis (IAP) and C-section birth with IAP) on the maturation of the infant gut microbiota.

### RESULTS

Supervised ordination method (tD-RDA and PERMANOVA) were used to test the hypothesis that mode of delivery and its associated clinical care practice (VD, VD\_IAP and CS) influence the bacterial colonization of the infant's gut. Dissimilarity in the bacterial community composition was determined using the Bray-Curtis distance.

### METHODOLOGY

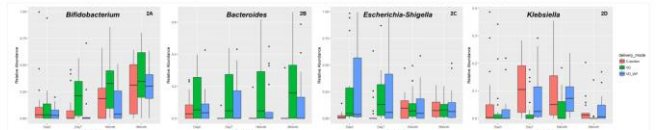
Three groups of healthy term born infants were recruited from the National University Hospital (Singapore): (1) Infants born vaginally (VD, n=24), (2) Infants born vaginally whose mothers received IAP (VD\_IAP, n=21) and (3) Infants born by C-section (CS, n=23). Intrapartum antibiotic prophylaxis was administered (1) to prevent neonatal group B Streptococcus (GBS) infection or post-cesarean maternal infection or (2) due to the development of complications during delivery. Stool samples (200 samples) were investigated at birth, day 7, 1 and 3 month to determine and monitor the maturation of the gut microbiota in early life using 16S rRNA sequencing. Multivariate data analysis (Distance based redundancy analysis (DbrDA) using Bray-Curtis distance and permutational multivariate analysis of variance (PERMANOVA)) was performed. Pairwise group comparisons were performed using non-parametric Mann-Whitney U test. Spearman's rank-order correlation was used to measure the strength and direction of ranked variables. Shannon diversity index was used to measure species diversity.



Figures 1A, 1B and 1C: Distance-based redundancy analysis of infant gut microbiota at day 7, 1 month and 3 month. The arrow vectors indicate the weight and direction of the least abundant genera. Labelled groups represent their respective centroids.

- Infants born vaginally (VD) harbored a bacterial community dissimilar from the CS and VD\_IAP groups, and this was statistically significant at day 7 (VD vs CS, p=0.001; VD vs VD\_IAP, p=0.04); 1 month (VD vs CS, p=0.003; VD vs VD\_IAP, p=0.000) and 3 month (VD vs CS, p=0.002).
- The bacterial community of CS and VD\_IAP groups were marginally dissimilar at week 1 (p=0.034) and similar at 1 and 3 month.

- Pairwise group comparisons showed that Bifidobacterium, Bacteroides and two members of the Enterobacteriaceae family, Escherichia-Shigella and Klebsiella, were the main bacterial groups that differentiate the infants' groups based on the mode of delivery and its associated clinical care practice (IAP), after adjusting for multiplicity (Benjamini-Hochberg).



Figures 2A, 2B, 2C and 2D: Relative abundance of Bifidobacterium, Bacteroides, Escherichia-Shigella and Klebsiella in the first three months of life.

- A delayed colonization by Bifidobacterium was observed in VD\_IAP and CS but not in VD born infants. This was statistically significant at day 7 (VD vs VD\_IAP, p=0.046; VD vs CS, p=0.011).
- CS born infants presented a delayed colonization by Bacteroides at day 7 (VD vs CS, p=0.001), 1 month (VD vs CS, p=0.004) and 3 month (VD vs CS, p=0.008).
- Escherichia-Shigella was higher in VD compared to CS and VD\_IAP at day 7 (VD vs CS, p=0.011; VD vs VD\_IAP, p=0.046).
- Klebsiella was higher in CS compared to VD at day 7 (VD vs CS, p=0.011), 1 month (VD vs CS, p=0.011) and 3 month (VD vs CS, p=0.001).
- Klebsiella was higher in VD\_IAP at 3 month (VD vs VD\_IAP, p=0.001).
- A negative correlation (Spearman's rank) between Bifidobacterium and Enterobacteriaceae was observed at day 7 (r=-0.356, p=0.004) and 1 month (r=-0.357, p=0.004).

- The effect of mode of delivery and its associated clinical care practice (IAP) on the species diversity was evaluated. The species diversity was higher in VD compared to VD\_IAP at day 7 (p=0.027). Interestingly, the species diversity in the meconium samples present larger inter-individual variation within the 3 groups and tend to be higher than in the subsequent stool samples at day 7, 1 month and 3 month.

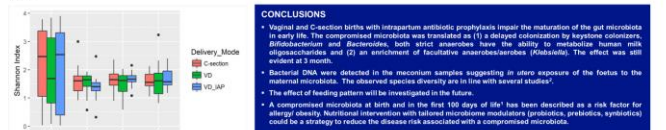


Figure 3: Species diversity in the first three months of life.

**CONCLUSIONS**

- Vaginal and C-section births with intrapartum antibiotic prophylaxis impair the maturation of the gut microbiota in early life. The compromised microbiota was translated as (1) a delayed colonization by Bifidobacterium, Bifidobacterium and Bacteroides, both strict anaerobes have the ability to metabolize human milk oligosaccharides and (2) an enrichment of facultative anaerobes/enterics (Klebsiella). The effect was still evident at 3 months.
- Bacterial DNA were detected in the meconium samples suggesting in utero exposure of the foetus to the maternal microbiota. The observed species diversity and its high inter-individual diversity could be a strategy to reduce the disease risk associated with a compromised microbiota.
- The effect of feeding pattern will be investigated in the future.
- A compromised microbiota at birth and in the first 100 days of life has been described as a risk factor for allergy, obesity, metabolic intervention with reduced mucosal modulators (probiotics, prebiotics, synbiotics).

**REFERENCES:**

1. Wei Wei Thwe Khine et al. 2017 Science Translational Medicine. Meta-Data Analysis of 2017 Journal of Allergy and Immunology. doi:10.1126/scitranslmed.2017.01111

2. T. Muller et al. 2017 Genes. Meta-Correlation of 2017. Scientific Reports. Link: Wang et al. 2017. Frontiers in Microbiology.

Unpublished  
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**FROM A PROTECTED ENVIRONMENT...**

**...TO A CHALLENGING EXTRA-UTERINE WORLD...**



**...Breast feeding is important for optimal development of infants**



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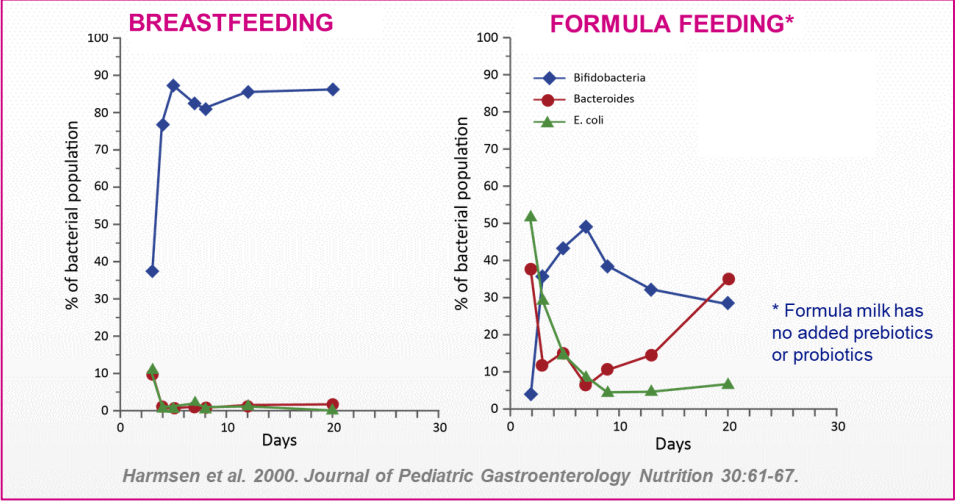


# HUMAN MILK CONTAINS MULTIPLE BIOACTIVE COMPOUNDS THAT IMPACT IMMUNE & MICROBIOTA DEVELOPMENT

Milk Constituent	Bioactive Component
Cells	Lymphocytes, Macrophages, Granulocytes
Proteins	Antibodies, Growth Factors, Cytokines
<b>Bacteria</b>	<i>Bifidobacterium</i> , Lactobacilli,
<b>Oligosaccharides</b>	(HMOs) ~1000 different
Fatty Acids	Saturated (45%), MUFA (40%),  PUFA (15%): 0.35% DHA 0.60% ARA
Protein, carbohydrates, others, macromolecules	Allergens, Lactose, Nucleotides
Minerals, Vitamins	Mg, Zn, Fe, Se, Vit A, C, E



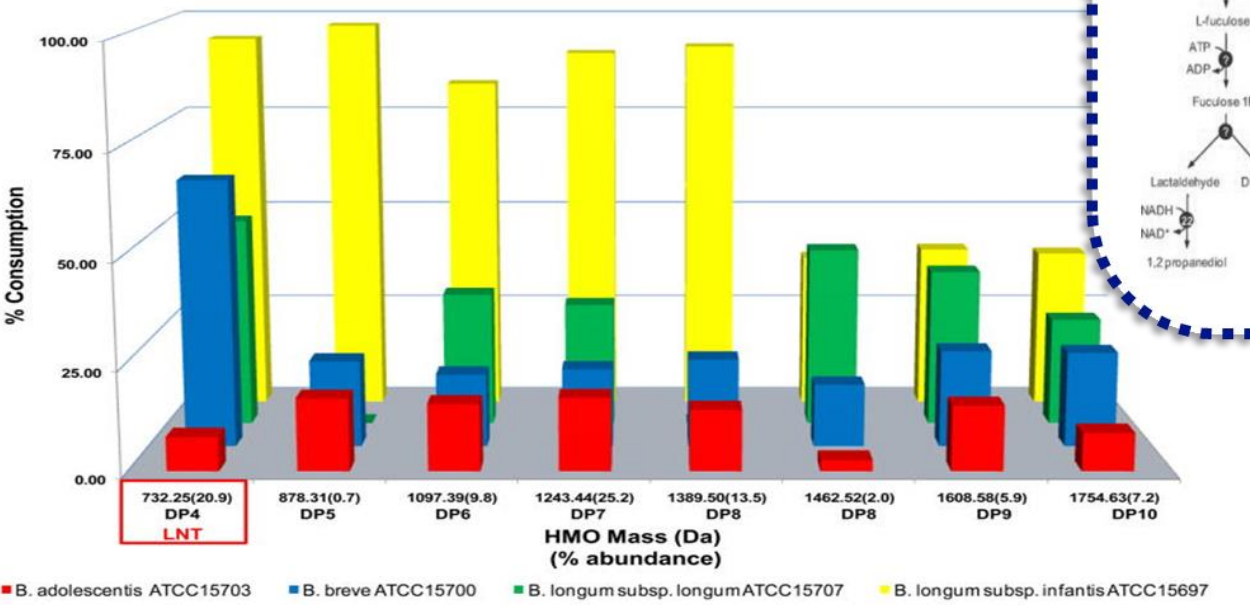
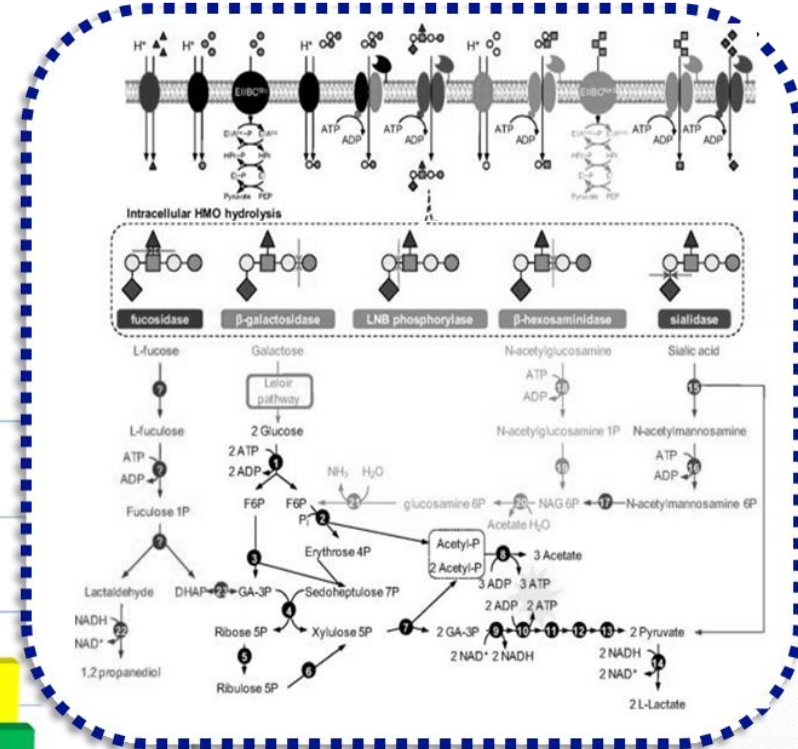
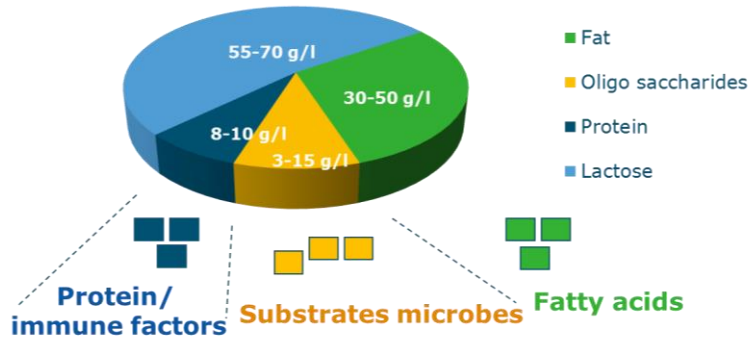
**Effect on microbiota composition- *Bifidobacterium* dominated microbiota in breastfed infants**



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# MILK CONTAINS GROWTH SUBSTRATES FOR BIFIDOBACTERIA



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# BIFIDOBACTERIA-DOMINATED COMMUNITY- BUT NOT ALL SPECIES ARE NATURALLY PRESENT IN THE INFANT GUT



Curr Microbiol (2017) 74:987–995  
DOI 10.1007/s00284-017-1272-4

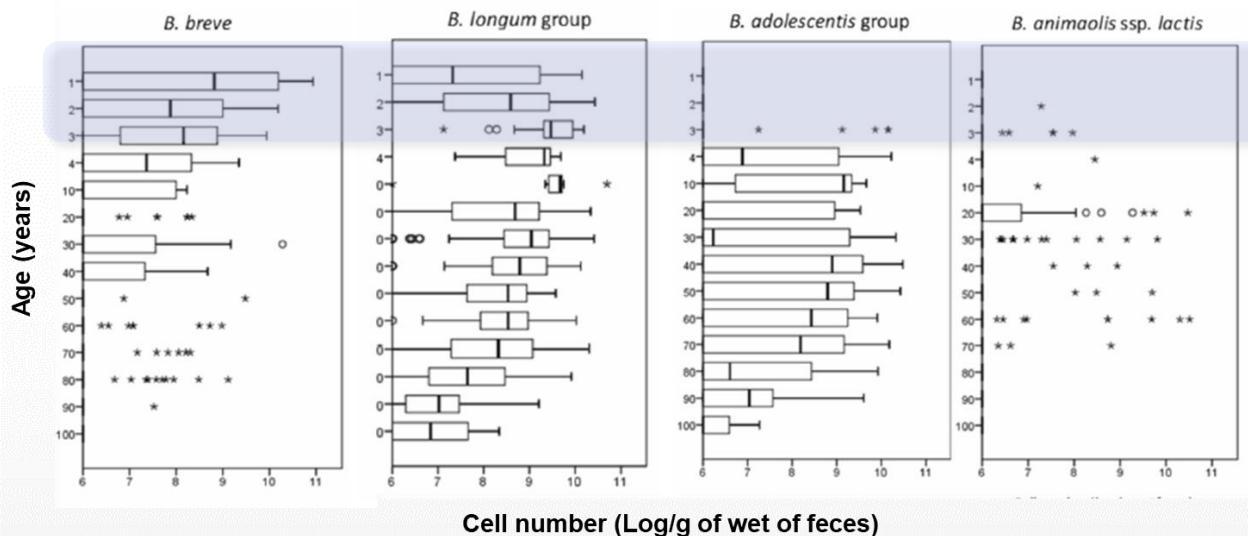


## Age-Related Changes in the Composition of Gut *Bifidobacterium* Species

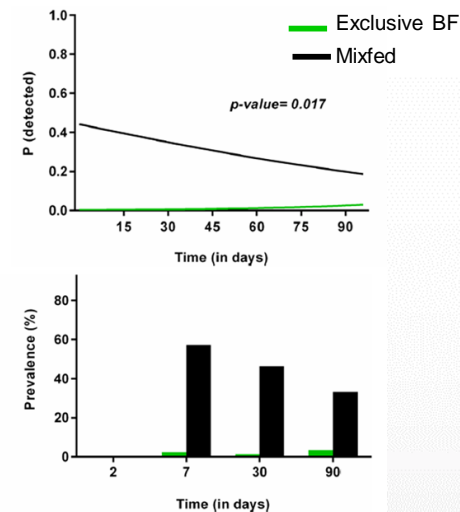
Kumiko Kato<sup>1</sup> · Toshitaka Odamaki<sup>1</sup> · Eri Mitsuyama<sup>1</sup> · Hirosuke Sugahara<sup>1</sup> · Jin-zhong Xiao<sup>1</sup> · Ro Osawa<sup>2</sup>

Early-Life Events, Including Mode of Delivery and Type of Feeding, Siblings and Gender, Shape the Developing Gut Microbiota

Rocio Martin<sup>1,4\*</sup>, Hiroshi Makino<sup>2,5\*</sup>, Aysun Cetinyurek Yavuz<sup>1</sup>, Kaouther Ben-Amor<sup>1</sup>, Mieke Roelofs<sup>1</sup>, Eiji Ishikawa<sup>2</sup>, Hiroyuki Kubota<sup>2</sup>, Sophie Swinkels<sup>1</sup>, Takafumi Sakai<sup>2</sup>, Kenji Oishi<sup>2</sup>, Akira Kushiro<sup>2</sup>, Jan Knol<sup>1,3</sup>



## *B. animalis* subsp. *lactis*



Supplemented *B. longum* subsp. *infantis* EVC001 (Frese et al., 2017) and *B. breve* M-16V (Chua et al., 2017) could stably colonize the infant gut- detected >1 month after the supplementation ceased

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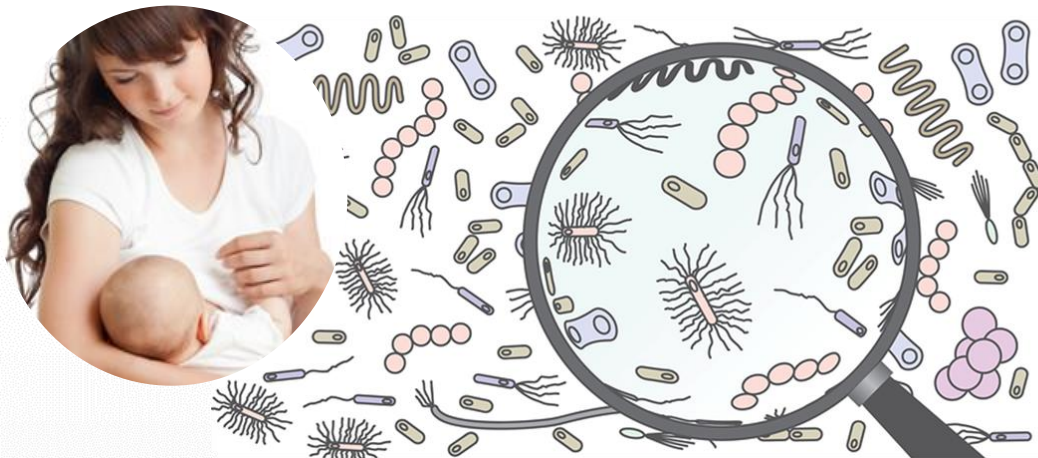




# HUMAN MILK CONTAINS BACTERIA



Human milk contains between  $10^3$  -  $10^5$  bacterial cells/ml



Milk microbiota is dominated by

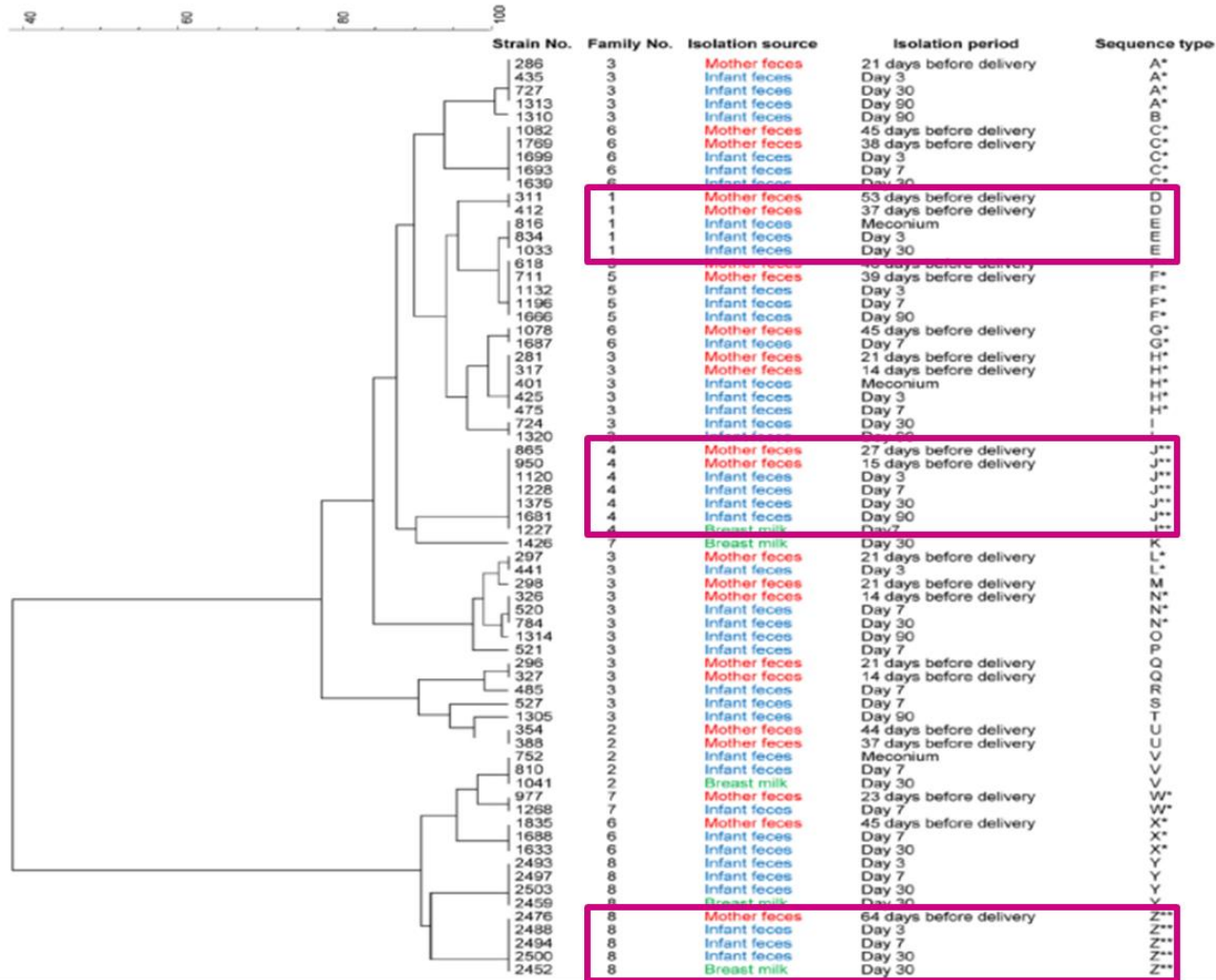
- *Staphylococcus*
- *Streptococcus*
- *Propionibacterium*
- *Bifidobacterium*
- *Lactobacillus*

*These bacteria are anticipated to protect the infant against infections and contribute to the maturation of the immune system*

Alba Boix-Amorós et al., 2016

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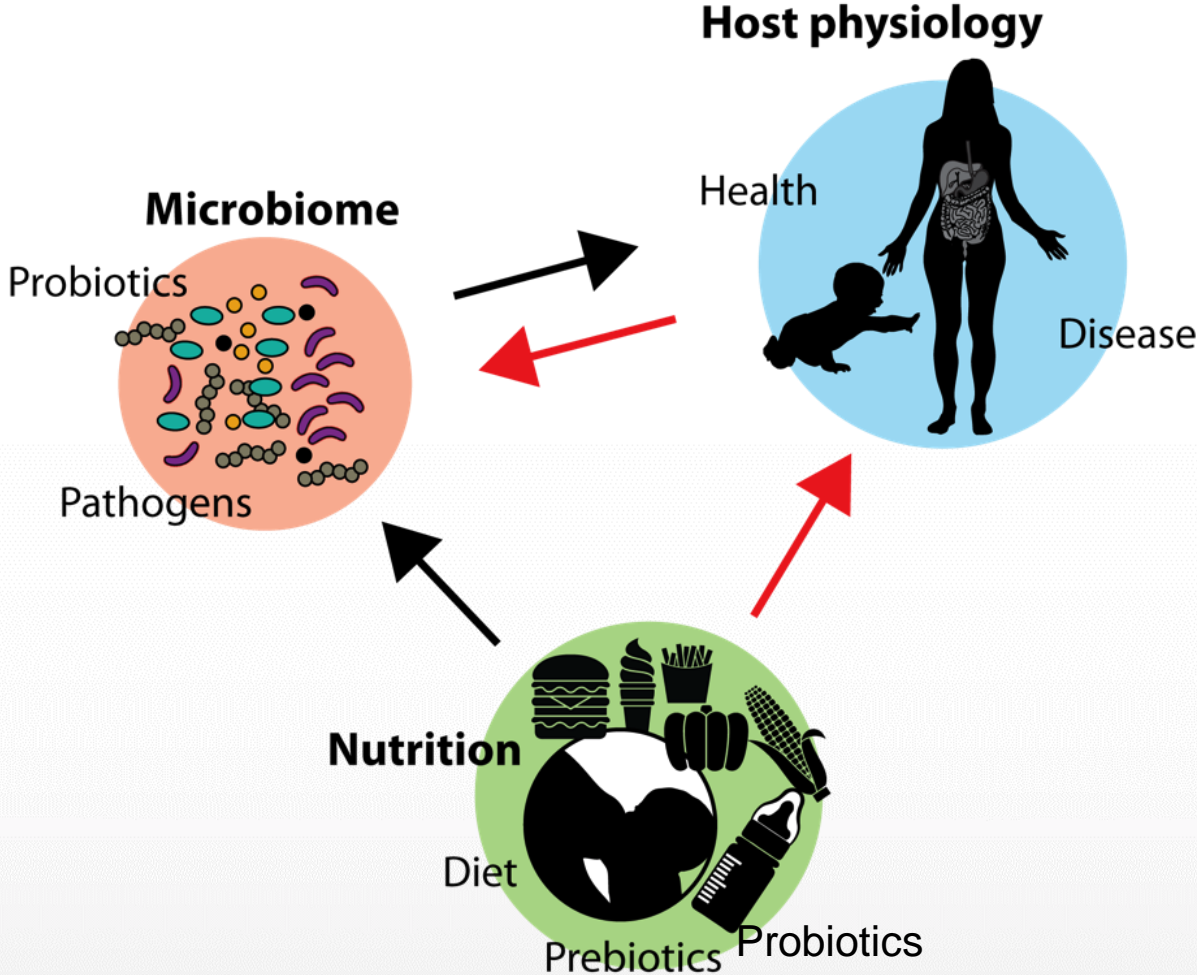
# TRANSMISSION OF MATERNAL MICROBIAL HERITAGE AT BIRTH AND DURING BREASTFEEDING



Makino H et al. Applied and Environmental Microbiology, 2011; 77 (19): 6788-6793

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# CAUSE AND EFFECT- THE ROLE OF NUTRITION TO REBALANCE AND SHAPE THE MICROBIOTA



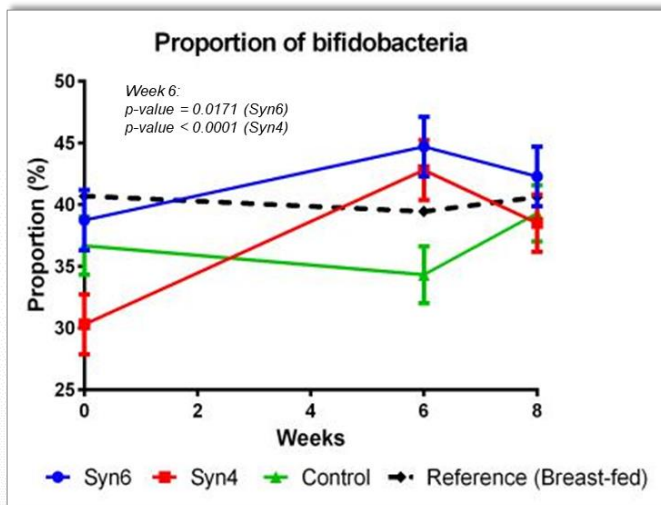
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# COLOR STUDY: SYNBIOTIC SUPPLEMENTATION INCREASES BIFIDOBACTERIA AND REDUCES *C. DIFFICILE* IN INFANTS



N= 290 Healthy infants, 6-19 weeks of age

\*Synbiotics: scGOS/lcFOS + *B. breve* M-16V at a dose of 10<sup>10</sup> CFU



Abundance of bifidobacteria in early life is a microbial indication of immune fitness and gut health  
(Hong PY et al, 2010).

*C. difficile* in infants

## BIFIDOGENIC EFFECTS OF A UNIQUE SYNBIOTIC MIXTURE (scGOS/lcFOS AND *B. BREVE*M-16V) IN HEALTHY INFANTS

DANONE NUTRICIA RESEARCH | NUTRICIA RESEARCH

Pheocher, N. I., Wang, S. F., Chorris, S. F., Irazola, S. F., Martin, R. J., Renteria, S. F., J. Volz, J. R. (2017) *Journal of Pediatric Gastroenterology and Nutrition*, 54(1), 104-111

\*Synbiotics: scGOS/lcFOS + *B. breve* M-16V at a dose of 10<sup>10</sup> CFU

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### INTRODUCTION

Human milk from healthy mothers is considered the optimal nutrition for infants and found to be nonsterile in the recent decade. Based on the culture method, it is estimated to contain about 10<sup>10</sup> bacterial cells/ml (1). *B. breve* is the most commonly isolated infant type bifidobacterium from human milk (2). It is one of the dominant species in the infant gut, involved in metabolization of human milk oligosaccharides (HMOs) and production of vitamins (3, 4) to support the healthy growth of the infants.

Bifidobacteria play key roles in early life gut health and immune maturation. However, not all infants are colonized by bifidobacterium species and some are even devoid of them (5). Environmental factors, such as mode of delivery, antibiotic administration and feeding patterns, influence the colonization process of gut microbes and the abundance of bifidobacteria in the infants' gut during early life. Given the benefits of the presence of bifidobacteria, it is important that an infant to be colonized with the right bifidobacteria to create the right gut environment for the infant's healthy growth. Synbiotics containing bifidobacterium species can be applied to increase the level of bifidobacteria in the infant's gut (6); however, effects of synbiotics on gut health are dose and strain dependent (7).

The aim of the study was to evaluate the bifidogenic effects of two different doses of synbiotics in healthy infants.

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### MATERIALS AND METHODS

An exploratory, multi-center randomized, double-blind, placebo-controlled study was conducted in Thailand to evaluate the bifidogenic effect of two different doses of synbiotics in 290 healthy infants aged from 2-8 months. All participating centres obtained approval of their independent local Clinical Review Board. Written informed consent was obtained from all parents before the study started.

All infants eligible in formula fed groups started a 2-week run-in period with non-hydrolyzed cow's milk-based formula (control formula). Infants who have successfully completed the run-in period, were randomized to receive either one of the two investigational formula or the control formula for a double-blind period of 8 weeks. Investigational formulas were non-hydrolyzed cow's milk-based formula supplemented with 0.5g/100 ml short chain galactooligosaccharides and long chain fructo-oligosaccharides (scGOS/lcFOS ratio 5:1) with *B. breve* M-16V at a dose of either 10<sup>10</sup> CFU/ml (Syn6) or 10<sup>9</sup> CFU/ml (Syn4). After the intervention period was completed, infants received control formula for a period of 2 weeks as the wash out period. Non-randomized infants who were exclusively breast-fed were included as a reference group (Fig 1).


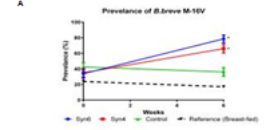
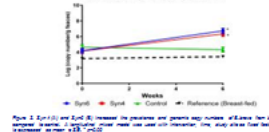




Fig 1 (continued) and Fig 2 (continued) show the prevalence and genomic copy numbers of *B. breve* M-16V in Syn6 and Syn4 groups compared to the control group.

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### CONCLUSION:

An infant-type Bifidobacterium, *B. breve* M-16V combined with scGOS/lcFOS (BFI) at a lower dose to the level of bacteria in human milk, increased infant type Bifidobacterium species in infants. This relatively low dose of viable bacteria may be a suitable approach to support the normal development of the gut microbiome in healthy infants during early life.

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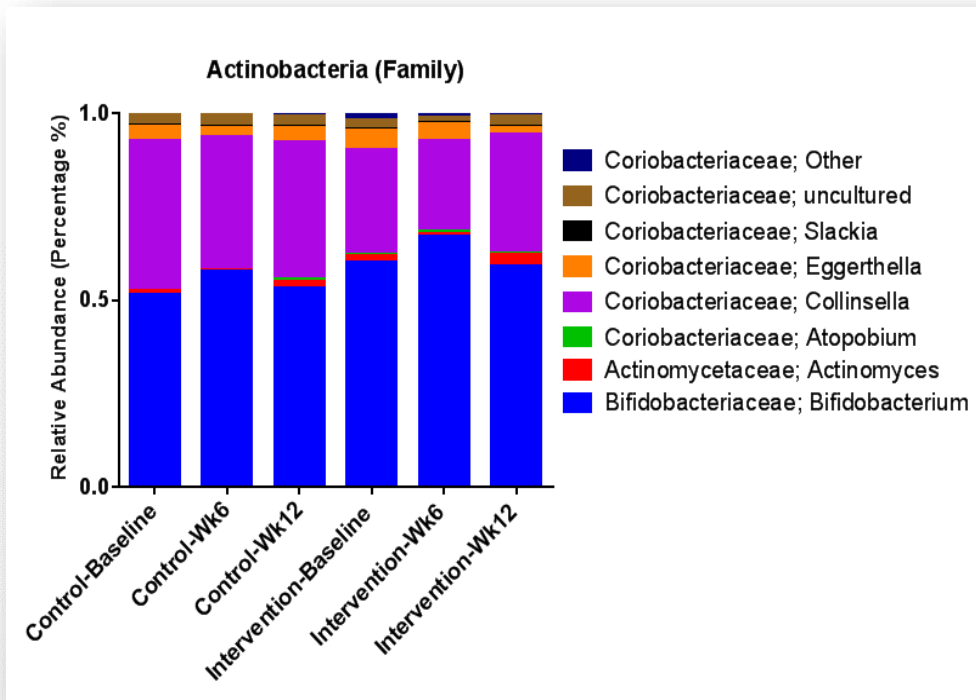


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# YCF SUPPLEMENTED WITH A SYNBIOTICS MIXTURE SIGNIFICANTLY INCREASED FECAL BIFIDOBACTERIA LEVEL IN HEALTHY YOUNG CHILDREN AGED 1-3 YEARS



N= 129 Healthy young children, 1-3 years



**GUT MICROBIOTA OF HEALTHY YOUNG THAI CHILDREN CONSUMING SYNBIOTICS SUPPLEMENTED FORMULA**

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**BACKGROUND AND RATIONALE**

As a child matures, there are gradual changes in dietary habits and environment resulting in constant stimulation of the gut. It is commonly hypothesized that the gut microbiota of young children is similar to that of an adult. However, recent studies suggested that the microbial profiles of young Caucasian children up to adolescence were still distinguished by their level of bifidobacteria. However, the impact of supplementation in young child formula on their gut microbiota composition is even less explored [1]. In Asian countries, there has been little studies have focused on establishing the profile of young children microbiota and even less reported on effect of intervention.

**METHODOLOGY**

This randomized, double-blind and controlled multi-centre clinical study was approved in Thailand by Ethics Committee and was registered in the International Clinical Registry (NCT02272) in 2015. Healthy subjects age ranging from 1-3 years old from Thailand were recruited for a 12-week intervention study. Subjects were randomized into two arms: synbiotic intervention (n=65) supplemented with aDOS (aDOS (2.1) at 1 g/100mL and 1.2 x 10<sup>10</sup> CFU/g Bifidobacterium breve M-16V) and control group (n=64) standard cows milk formula.

Faecal samples were collected at baseline (before intervention), at week 6 and week 12 and processed using QIAzol Lysis kit with modified bead-beating protocol. The microbial composition of the samples were analysed by qPCR targeting specifically Bifidobacterium species and 16S V3/V4 ribosomal RNA gene (sequencing using primer optimized by Sm et al [2]) to provide insights into the microbial community over time. On the basis of all measurements from baseline to week 12, the p-values (false discovery rate) were also estimated [2].

**RESULTS**

The overview of the gut microbiota composition generated by 16S rDNA sequencing indicated that the dominant bacterial phyla were Firmicutes and Actinobacteria within the cohort.

When comparing baseline to Week 12, there was a statistically significant increase of Bifidobacterium in both groups (p=2.7x10<sup>-5</sup> in control arm; p=2.5x10<sup>-5</sup>, p=1.4x10<sup>-4</sup> in synbiotic arm; Wilcoxon Signed Rank test). The same comparison showed no statistically significant difference within each intervention group for Bifidobacteriaceae Family (p=0.62 in control arm; p=0.24 in synbiotic arm; Wilcoxon Signed Rank test).

Using qPCR, there was an increase in B. breve absolute gene count over the 12 weeks intervention in the active group compared to control (Figure 2A), p=0.014 and p=0.004, at week 6 and week 12, respectively. Mann-Whitney U test) across all baseline (p=0.044). An increase of B. breve M-16V absolute gene count was also observed in the active group compared to control (p=0.002 and p=0.001, at week 6 and week 12, respectively; Mann-Whitney U test) across all baseline (p=0.002). The prevalence of B. breve M-16V was 21.2% at baseline, 22.2% at week 12 in the active group while the prevalence of B. breve was 50.5% at baseline, 52.5% at week 6 and 52.5% at week 12 in synbiotic group (Figure 2B). This observation may indicate that synbiotic supplementation sustains endogenous B. breve. Regarding other Bifidobacterium species, there were no significant differences within and between the groups across the whole intervention period, which indicated B. breve M-16V supplementation did not affect other Bifidobacterium species that were measured here.

**DISCUSSION**

We found that the microbiota of the age is at unique transition of microbial profiles. Notably, bifidobacteria were which were commonly accepted to reduce remained high. This may suggest functional importance of this bacteria group in the establishment of more adult like microbiota in the gut. The introduction of synbiotic increased composition of B. breve (not just B. breve M-16V) without affecting other bifidobacteria species, suggests robustness of the ecosystem to promote endogenous strains.

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**CONCLUSION**

Young child formula (YCF) supplemented with a synbiotic mixture significantly increased fecal bifidobacteria level in healthy young children aged 1-3 years. However, the influence of bifidobacteria on other beneficial bacteria in this microbial ecosystem has to be further investigated.

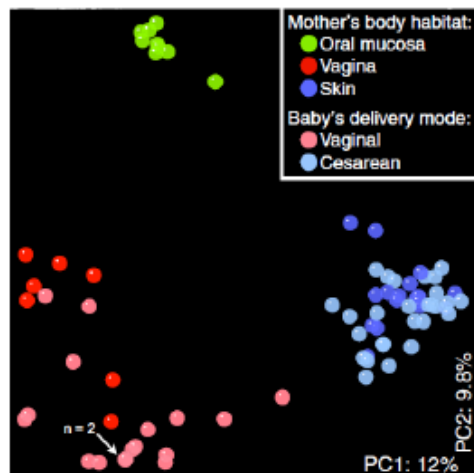
Kosuwon et al., *Beneficial Microbes* 2018 Apr 10:1-12. doi: 10.3920/BM2017.0110.

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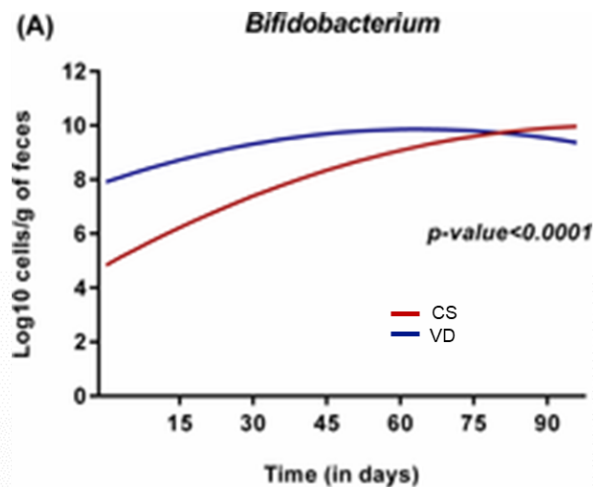
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# DIFFERENT MICROBIOTA COMPOSITION IN C-SECTION BORN INFANTS MAY EXPLAIN HIGHER RISK OF ALLERGY



Dominguez-Bello et al, 2010, PNAS 107: 11971-11975



Martin et al., 2016, PlosONE 2016, 11(6)

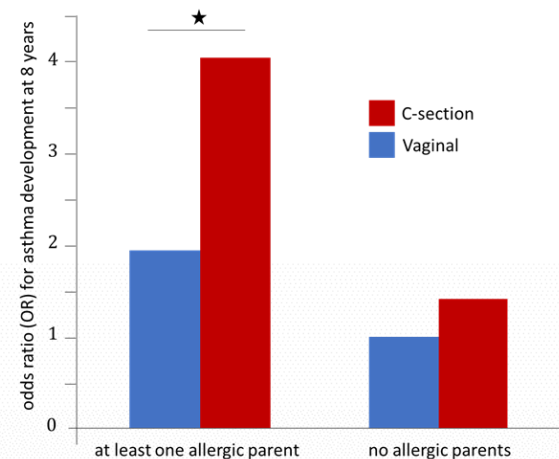
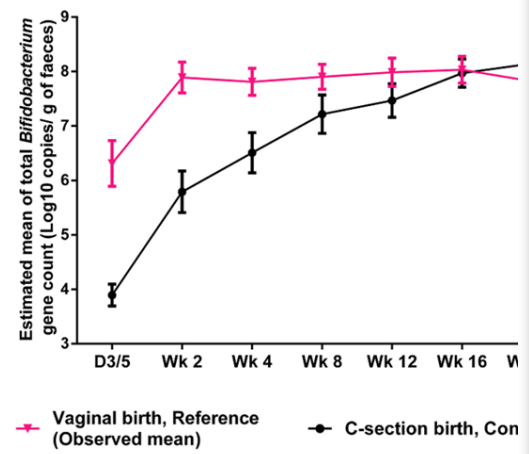


Figure based on Roduit et al., 2009, Thorax 64:107-113

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# SYNBIOTIC RESTORE THE DELAYED COLONIZATION BY BIFIDOBACTERIUM IN CS BORN INFANTS FROM THE FIRST DAYS OF LIFE

Restores the numk  
Restores the delayed colonis



### A synbiotic mixture of scGOS/lcFOS and *Bifidobacterium breve* M-16V restores the delayed colonization by *Bifidobacterium* observed in C-section delivered infants

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<sup>1</sup>101 Women and Children Hospital, Department of Paediatrics, Singapore; <sup>2</sup>Danone Nutricia Research, Singapore; <sup>3</sup>Danone Nutricia Research, Utrecht, The Netherlands; <sup>4</sup>101 Chulalongkorn Memorial Hospital, Department of Pediatrics and Neonatology, Bangkok, Thailand; <sup>5</sup>101 Chulalongkorn Memorial Hospital, Department of Pediatrics, Bangkok, Thailand

**BACKGROUND AND RATIONALE**  
Infants born by C-section miss the exposure to the maternal vaginal microbiota and the absence of microbial inoculation has been associated with a delayed colonization by commensal bacteria such as bifidobacteria. Epidemiological data have identified C-section birth as a risk factor for the development of immune and metabolic disorders. The objective of this study was to determine the effect of a specific synbiotic mixture of short-chain galacto-oligosaccharides, long-chain fructo-oligosaccharides (scGOS/lcFOS, ratio 2:1) and *Bifidobacterium breve* M-16V in restoring the delayed colonization by bifidobacteria observed in term infants delivered by elective C-section.

**METHODOLOGY**  
In a multi-country, double-blind, randomized controlled study, 153 infants born by C-section were randomly assigned to receive an infant formula supplemented with (1) scGOS/lcFOS (0.5g/100ml) and *B. breve* M-16V (1.2 x 10<sup>10</sup> CFU/100ml) (synbiotic group), or (2) scGOS/lcFOS (0.5g/100ml) (prebiotic group), or (3) a standard infant formula (control group) from birth until the age of 18 weeks. Infants delivered vaginally and breastfed as long as possible (N=20) were included as the reference group. Stool samples were collected at day 2 and/or day 5, week 2, week 4, week 8, week 12, week 16, and week 22 (5 weeks post-intervention). *Bifidobacteria*, *B. breve* and *B. breve* M-16V were analyzed by q-PCR. Faecal pH and short chain fatty acids (SCFA) were assessed. A generalized linear mixed model with regional distribution and identity link function was used and intervention effects were compared using a test. Safety and tolerance parameters were recorded in the diary on a weekly basis. Adverse events (AEs) of skin disorders were compared between treatment groups according to related family history of allergies using logistic regression.

**RESULTS**  
Out of the 153 infants randomized, 132 infants from the modified intention-to-treat (mITT) set were included in the data analysis with 45, 35, and 45 infants in the synbiotic, prebiotic and control groups, respectively. The mITT set consisted of all randomized subjects who provided at least one baseline and post-treatment stool sample. The reference group consisted of 25 infants.

The results showed the following:

- Delayed colonization by bifidobacteria in C-section delivered infants from the first days of life as compared to the vaginally delivered infants (Fig. 1A).
- Significantly higher level of bifidobacteria from the first days of life ( $p < 0.0001$ ) in the synbiotic as compared to the control group. This bifidogenic effect remained significant at week 12 ( $p < 0.001$ ), week 16 ( $p < 0.01$ ) and week 22 ( $p < 0.001$ ) (Fig. 1B).
- B. breve* was mainly detected in the synbiotic group (Fig. 2A). At week 22, *B. breve* M-16V was detected in 20% of the infants in the synbiotic group indicating the persistence of the probiotic strain 6 weeks post-intervention (Fig. 2B).

Figure 1: Delayed colonization by bifidobacteria in C-section delivered infants and effect of the intervention on the bifidobacteria population as determined by q-PCR. (A) Data expressed as estimated mean of total bifidobacterium gene count (Log10 copies/g of faeces).

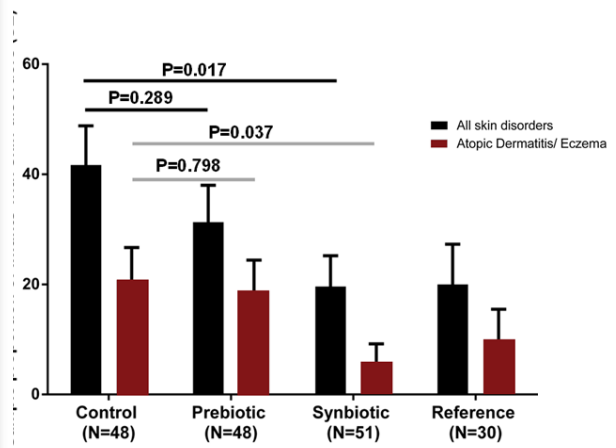
Figure 2: Effect of the intervention on the bifidobacteria (B) and *B. breve* (C). Data expressed as estimated mean (SD) and expressed in colony forming units (CFU).

**CONCLUSION**  
Supplementation with a specific synbiotic mixture of scGOS/lcFOS and *B. breve* M-16V in C-section delivered infants

- Compensates the delayed colonization by *Bifidobacterium* from the first days of life
- Emulates the gut physiological conditions (production of acetate and modulation of an acidic gut milieu) observed in vaginally born infants

These biological phenomena have been depicted as an indicator of gut health.

tential protective effect\* on skin rash especially eczema in early life



Less incidence of reported skin related disorders & eczema during the study

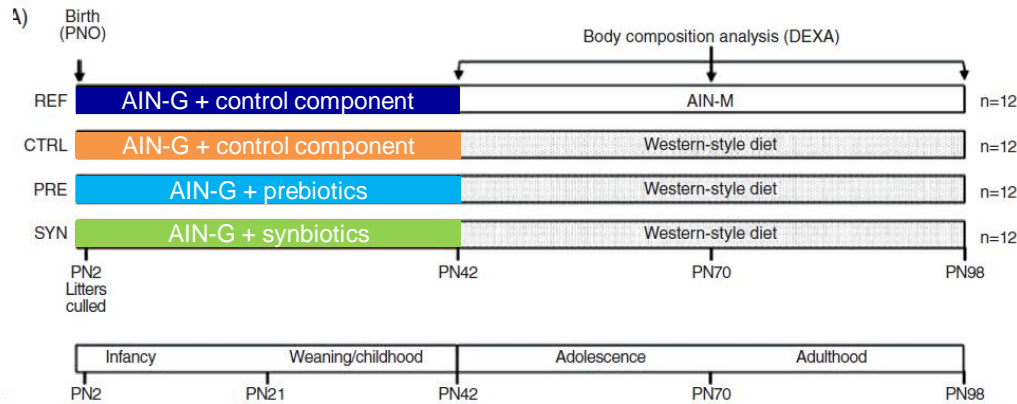
Chua MC et al.. JPGN 2017;65: 102–106

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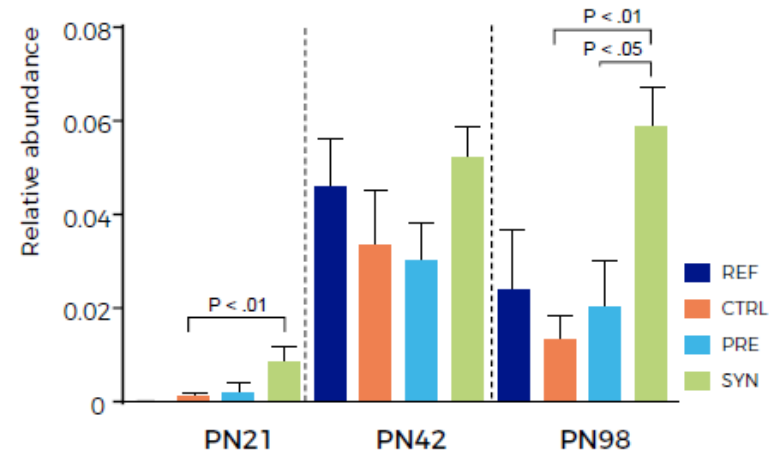
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# EARLY LIFE SYNBIOTIC SUPPLEMENTATION PROTECT AGAINST DIET-INDUCED OBESITY IN ADULT MICE



Synbiotic supplementation increased the abundance of *Bifidobacterium*



REF and CTRL: AIN-G\*

PRE: AIN-G + prebiotics (*scGOS/lcFOS*, 9:1)

SYN: AIN-G + synbiotics (*scGOS/lcFOS*, 9:1 + *B. breve M-16V*)

\*AIN-G (standard semi-synthetic diet appropriate for breeding) plus control component (maltodextrin)

Food intake did not differ between groups

Mischke M et al. *Diabetes Obes Metab.* 2018 Feb 20. doi: 10.1111/dom.13240.

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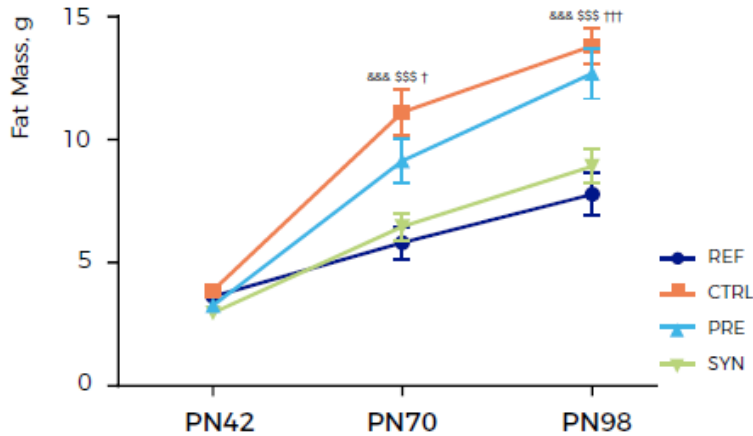


# EARLY LIFE SYNBIOTIC SUPPLEMENTATION PROTECT AGAINST DIET-INDUCED OBESITY IN ADULT MICE

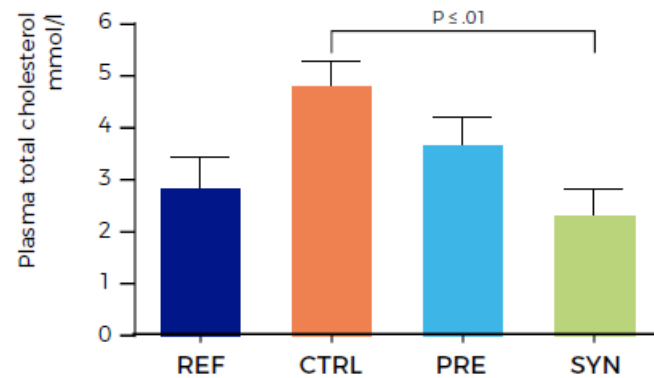
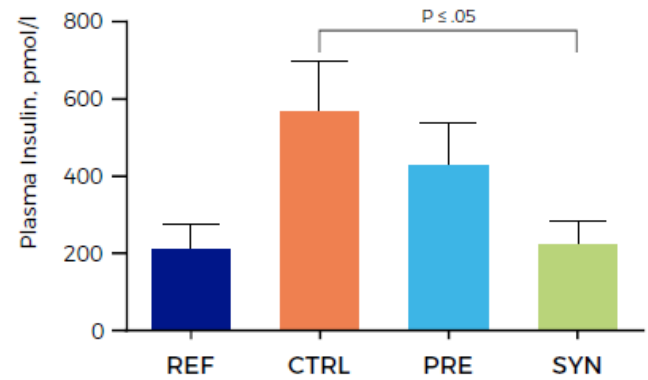


Provided long-term protection against diet-induced excessive fat accumulation

Improved insulin sensitivity and dyslipidaemia in adulthood



<sup>\$\$\$</sup> $P \leq .001$  indicates significance for REF vs CTRL  
<sup>††</sup> $P \leq .01$ , <sup>†††</sup> $P \leq .001$  indicate significance for PRE vs SYN.  
<sup>\$</sup> $P \leq .05$ , <sup>\$\$</sup> $P \leq .01$ , <sup>\$\$\$</sup> $P \leq .001$  indicate significance for CTRL vs SYN.



Mischke M et al. *Diabetes Obes Metab.* 2018 Feb 20. doi: 10.1111/dom.13240.

# TAKE HOME MESSAGE

- ★ **Microbial colonization following birth, is essential for establishing a symbiosis with our immune system and profoundly influences health throughout life**
- ★ **Breastfeeding and C-section delivered are key factors in early life that may impact long life health**
- ★ **Dysbiosis or altered microbial colonization is associated with the development of allergies**
- ★ **Nutrition represents a fundamental basis of the strong relationship between the gut microbiota, the immune system and health**
- ★ **Specific pre, pro and synbiotic interventions in early life successfully modulate the early microbial colonization and influences immune development.**

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# THANK YOU

DANONE NUTRICIA RESEARCH TEAM

The Netherlands

Singapore

...and many paediatricians,

Parents

and infants



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