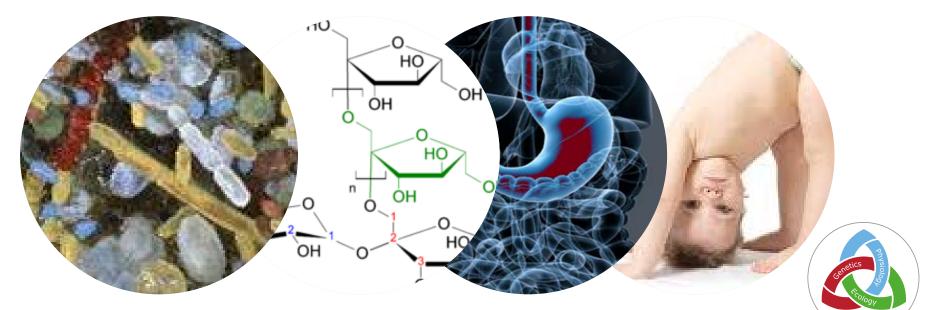
How to (not) groom our guts Early life microbiome dynamics and consequences throughout life

November 30th, 2017, Hauke Smidt

CarboHealth Symposium, Zwolle, NL



Laboratory of Microbiology

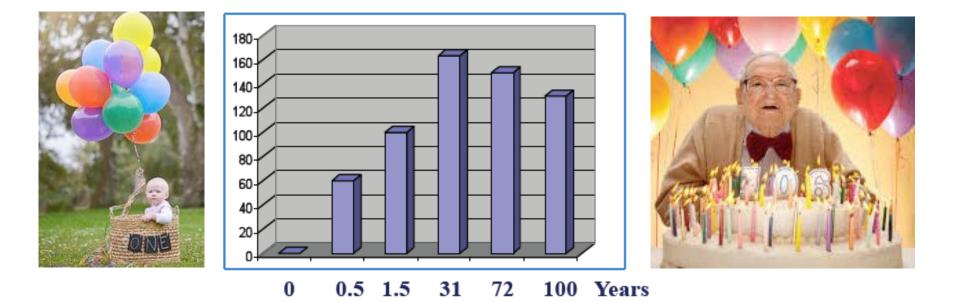


Growing up in a microbial world

- Importance of 'pioneer' species received from the mother
- Transmission of microorganisms from the maternal vagina and lower intestinal tract
- Infants born by Caesarean section are predisposed to development of allergies and asthma later in life
- Pre-maturely born infants are more susceptible to infections and have insufficient energy uptake



Microbial diversity throughout life

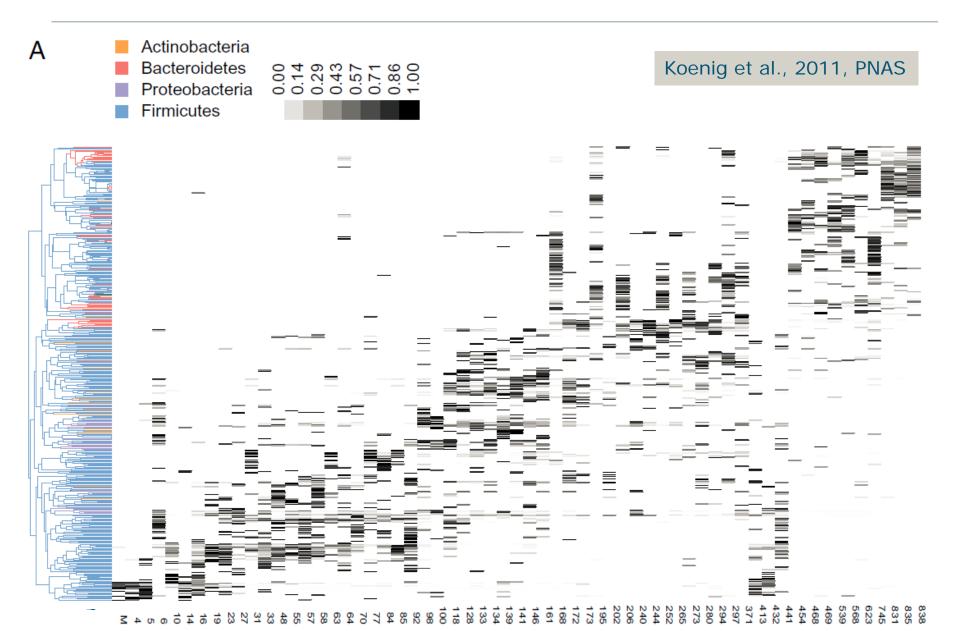


Born Sterile - Less Diversity at Older Age

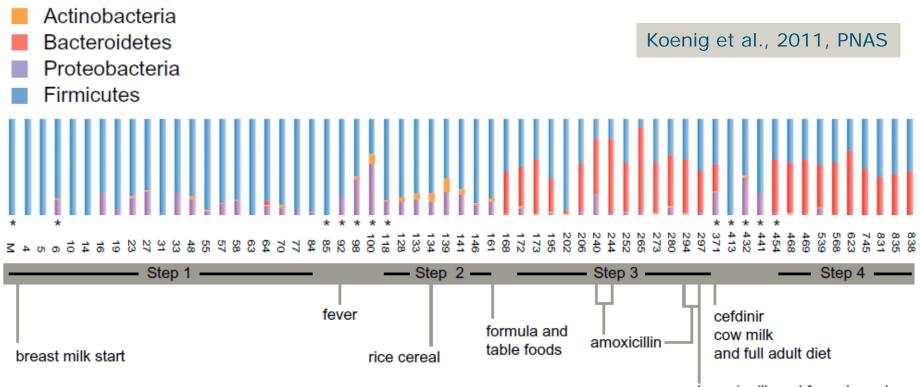
High Microbial Diversity Provides Resilience

Biagi et al PLoS One 2010

Microbial succession in newborns (human)



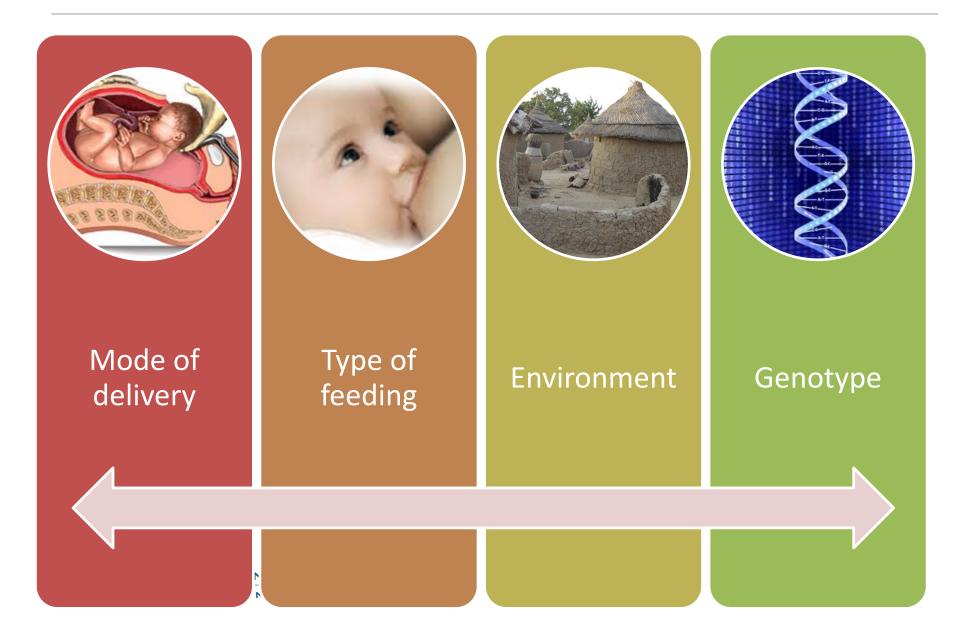
Microbial succession in newborns (human)



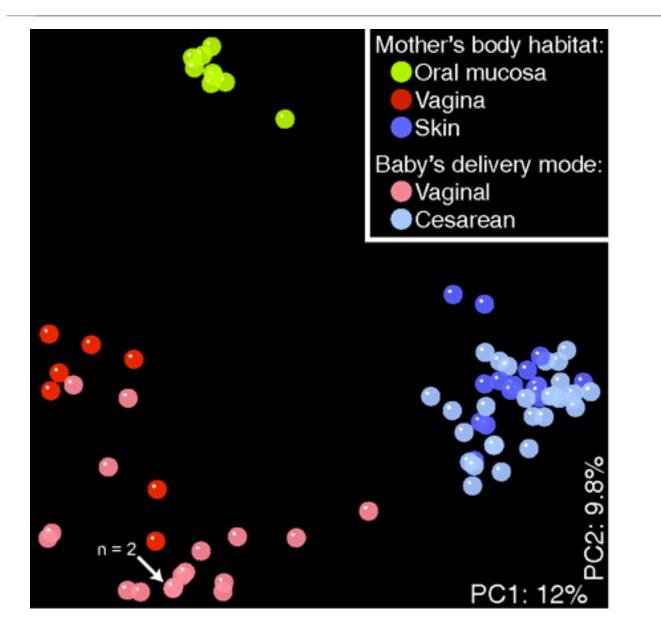
breast milk and formula end

- abrupt shifts of abundance taxonomic groups corresponding to changes in diet or health
 earliest microbiome enriched in genes facilitating lactate utilization
- •functional genes involved in plant polysaccharide metabolism present before the introduction of solid food
- •table foods increased he abundance of Bacteroidetes
 - Very low bifidobacteria in US Babies: Likely Technical: DNA Extraction / Primers etc

Many factors can affect microbiota



Delivery Mode Affects Colonization (Few Days)

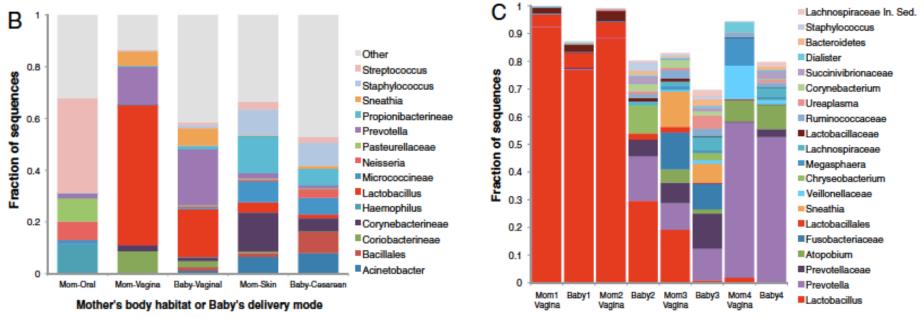


Dominguez-Bello et al. (2010) PNAS 11971–11975 Delivery Mode Affects Colonization (Few Days)

Infants born by Caesarean section are predisposed to development of allergies and asthma



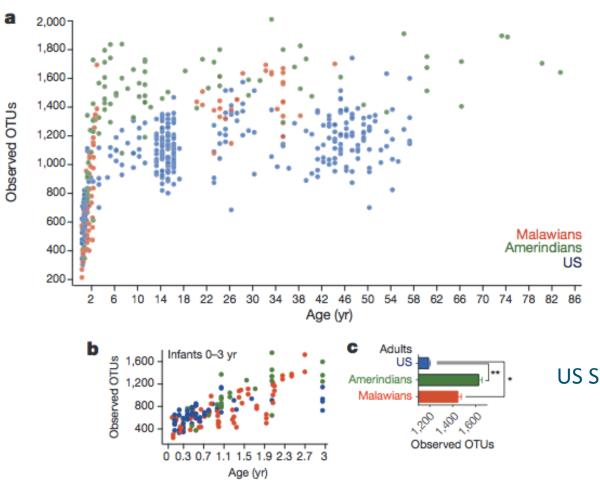
Normal Delivery



Mother's vagina or Vaginally-delivered baby

Gut microbiome across age and geography

Different socio-economic, geographic and cultural settings:



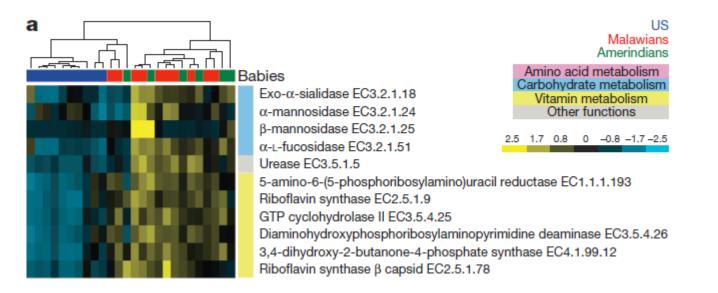
Amazonas of Venezuela rural Malawi US metropolitan areas

US Subjects Reduced Richness...

Yatsunenko et al., Nature 2012

Gut microbiome across age and geography

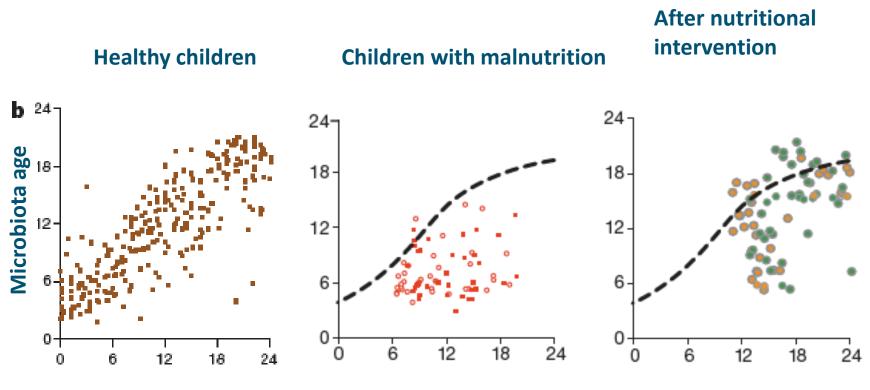
Differences not only in composition, but also function



Malawians and Amerindians have higher expression of genes related to vitamin and carbohydrate metabolism

Yatsunenko et al., Nature 2012

Malnutrition retards microbial development



chronological age (months)

Effects relative abundance of *Streptococcaceae* (up) and *Enterobacteriaceae* (down)
Way to characterize malnourished states, and responses to food interventions
Improvements in microbiota maturity were not sustained

Effects of Food - Glycans shape our microbiota

•<u>Dietary glycans:</u> – plants

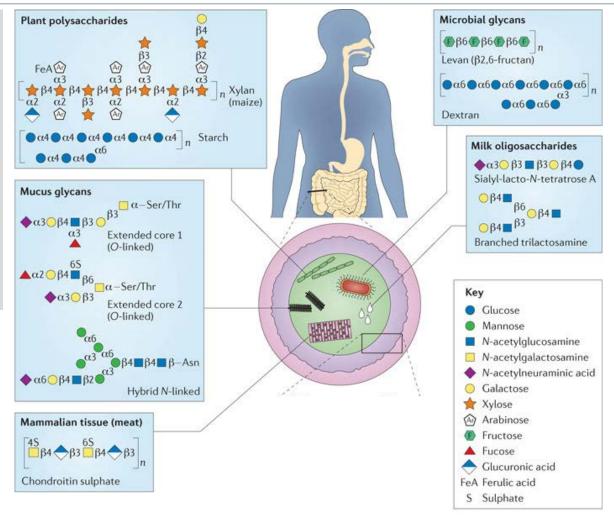
•Animal-derived:

- tissue

•Endogenous glycans:

- Mucus

Milk
 Oligosaccharides



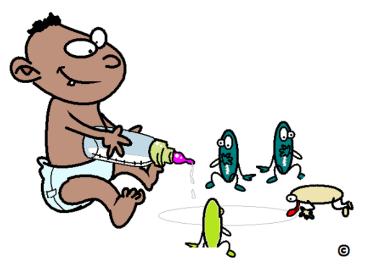
Koropatkin et al., 2012 Nature Reviews | Microbiology

Microbial transformation of indigestible glycans into SCFA
 → Nutrients for colonocytes and other gut epithelial cells

How does early life nutrition affect dynamics of microbial composition / function?

Do 2003 & 2016 formulas differ in nourishing the same gut bacteria as breastmilk?

Two Case Studies – KOALA (2003) & BINGO (2016)



Analysis of faecal microbiota composition by Illumina HiSeq of 16S rRNA genes

KOALA (2001 – 2003)



- Birth cohort study "Child, parents and health: lifestyle and genetic constitution"
- 2500 children followed from pregnancy to adulthood
- two major themes: allergy and asthma, and growth and development

210 fecal samples from 1-month old, breastfed and formula fed infants born in 2002-2003

- 124 Breastfed (BF)
- 103 Formulafed (FF)
- 15 Mixed (MF)

146 mother-infant pairs with matching milk/faecal sample 14

BINGO (2015 – 2016)



Radboud University

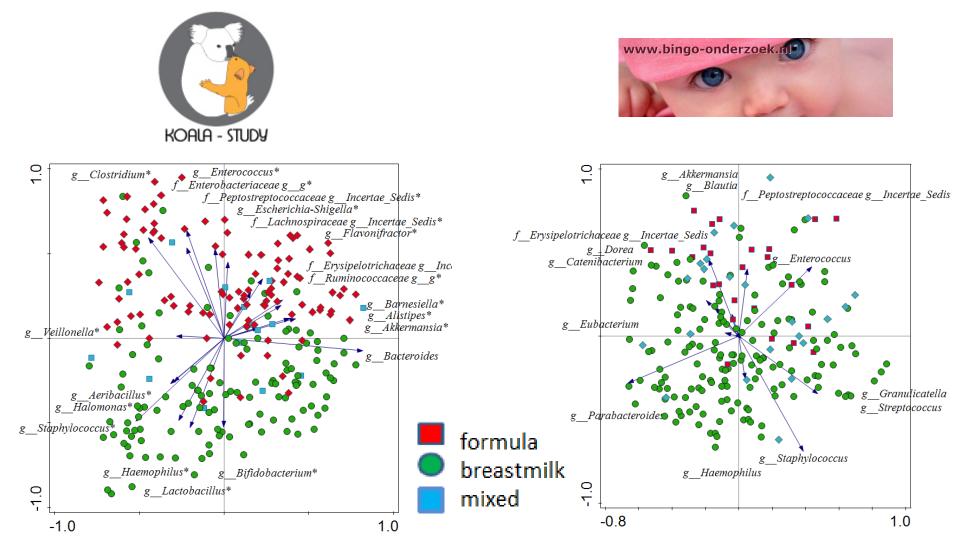


- Birth cohort study on stress, breastmilk quality and infant crying.
- 80 children born between 2015-2016, followed from pregnancy
- Milk & faecal samples collected 2, 6 and 12 weeks of age

242 Fecal samples

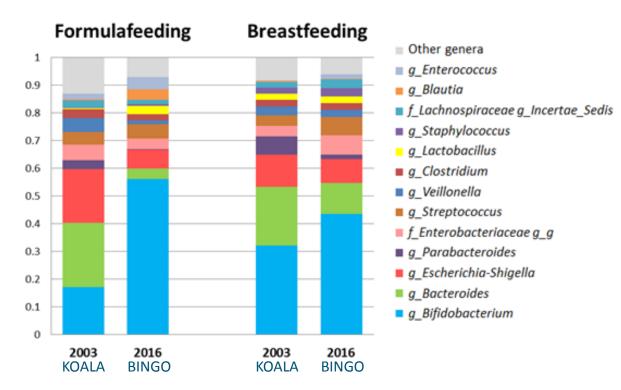
- 157 Breastfed (BF)
- 25 Formulafed (FF)
- 23 Mixed (MF)

Samples group by feeding mode (PCA)



But less so in 2016 vs. 2003

2003 vs. 2016 baby formula



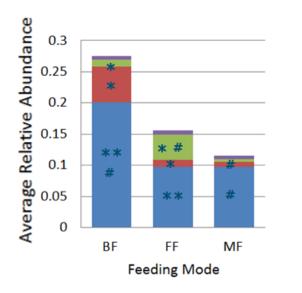
Fortified 2016 formulas:

Increase in *Bifidobacterium*, *Lactobacillus* Reduction in *Bacteroides* and *Escherichia- Shigella* group.

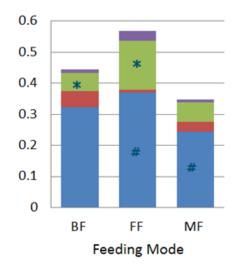
Fecal microbiota of 2016 FF and BF babies is more alike (Pearson correlation = 0.97) than in 2003 (Pearson correlation = 0.89)

The effect of formula on specific bifidobacterial phylotypes (OTUs)

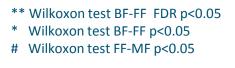
KOALA Cohort



BINGO Cohort



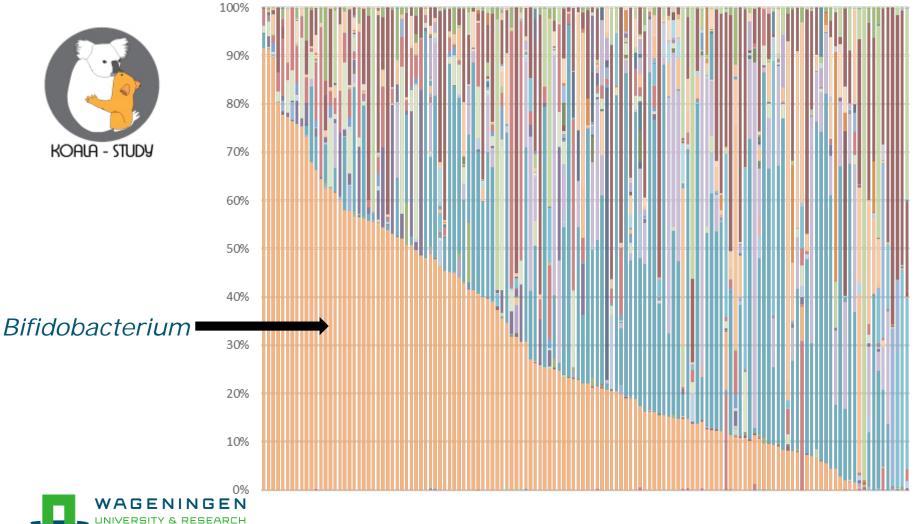
Other Bifidobacterium
 g_Bifidobacterium_OTU_L1
 g_Bifidobacterium_OTU_B1
 g_Bifidobacterium_OTU_L2



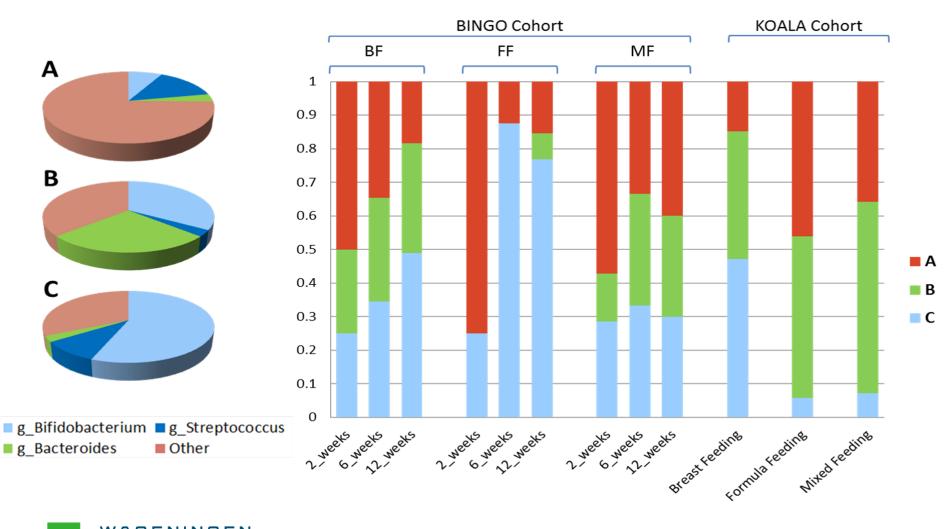
- BF-dominant Bifidobacterium OTUs
- less dominant Bifidobacterium OTUs 1
- major *Bifidobacterium* groups = BF
- less dominant Bifidobacterium OTUs 个
 - 9 low abundance taxa from 0.1% to 1% (FDR p<0.05)

High inter-individual variation in 4-week breastfed infants





DMM Clustering of Samples - "Baby enterotypes"

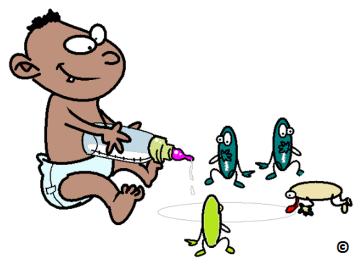




Two Case Studies – KOALA & BINGO

Can we link observed microbiota patterns to:

- Human milk oligosaccharides
- HMO degradation patterns in faeces

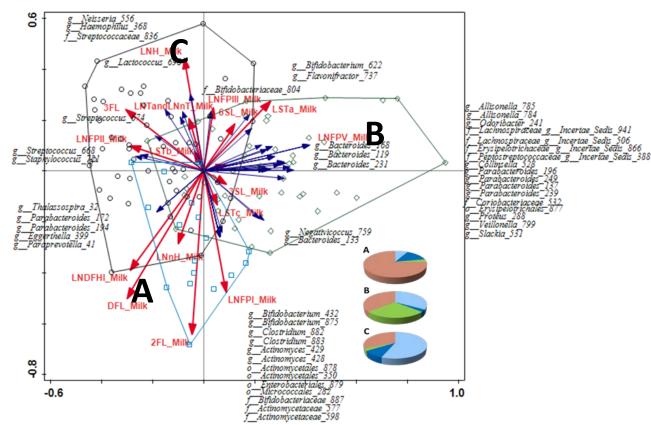


Analysis of faecal microbiota composition by Illumina HiSeq of 16S rRNA genes

Analysis of HMO composition in milk & faeces

Minor correlation HMO & microbiota

RDA with covariates

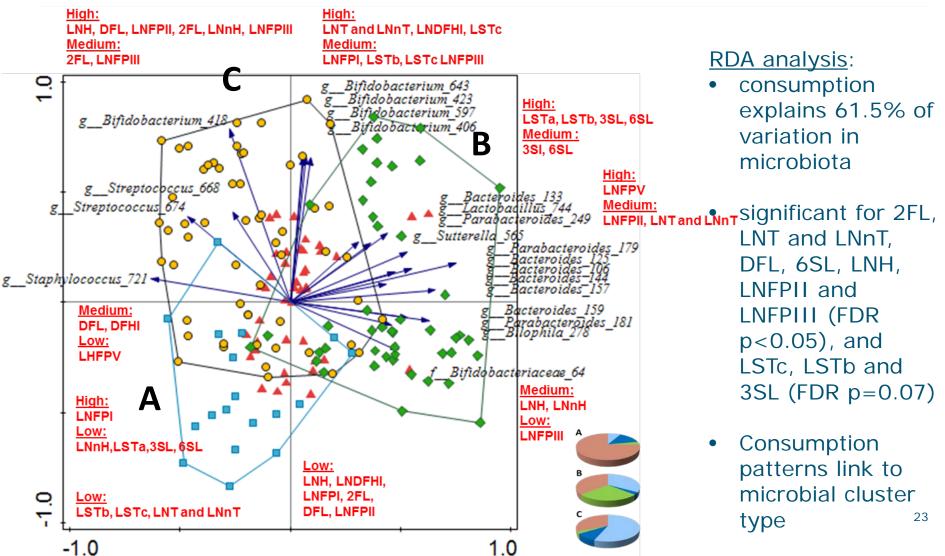


- Milk HMOs explain 14.2% variation in faecal microbial data
- No significance of HMOs based on FDR adjusted p-values (FDR p >0.05)
- HMO composition weakly associated with cluster type (FDR p=0.06)



Strong correlation HMO consumption & microbiota

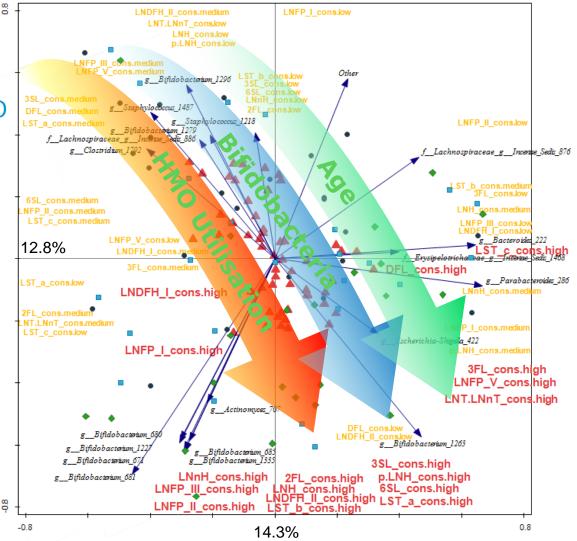




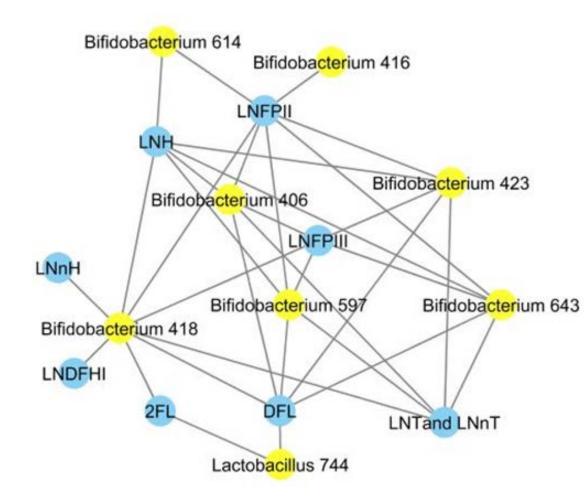
Strong correlation HMO consumption & microbiota



- Utilisation of the major HMO types in breastmilk increases with infant age
- High utilisation linked with high abundance of bifidobacteria



Bifidobacteria as key players in efficient HMO degradation



Bifidobacterium OTUs enriched in high consumers

They can be specialists or generalists for degradation of different HMOs

No significant difference in *Bacteroides* RA between high and low consumption groups for any of the HMOs

Conclusions

- Different stages during early life microbial dynamics towards *Bifidobacterium*-dominated communities
- Fortified infant formulas result in higher faecal Bifidobacterium abundance with community patterns more similar to those found in BF infants
- Bifidobacterium-dominated community types show most efficient degradation of HMOs
- Individual- and age-dependent differences in microbiota composition & function

The Team



Klaudyna Borewicz – Microbiology

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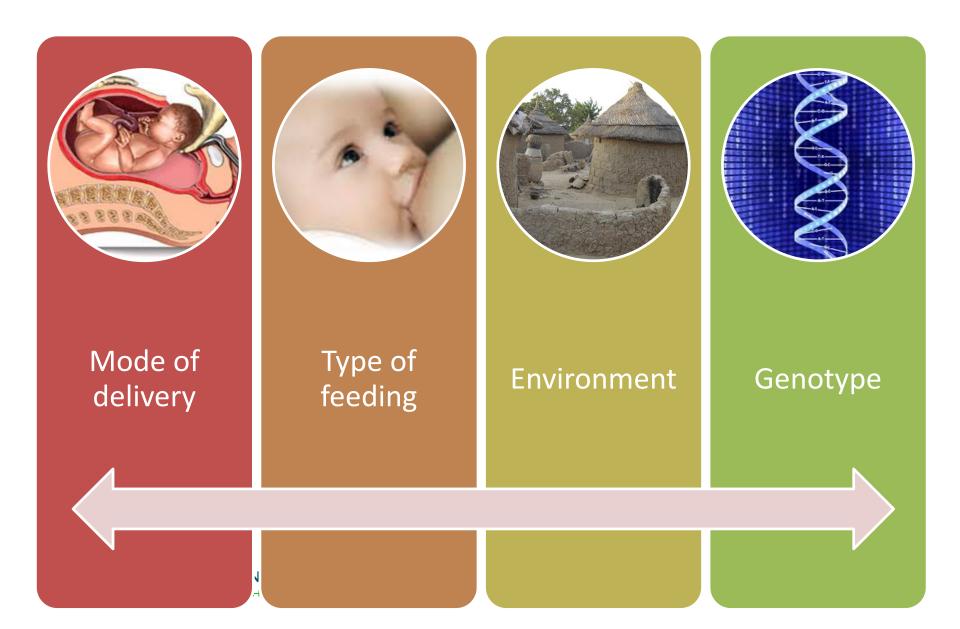
Ilja Arts John Penders Carel Thijs



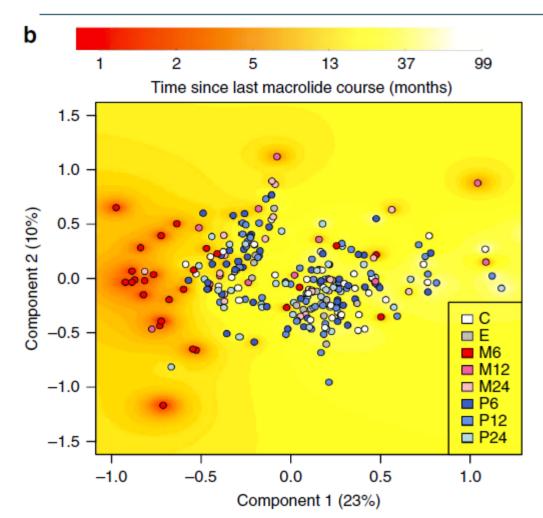




Many factors can affect microbiota



Antibiotic use affects microbiota composition & function in pre-school children

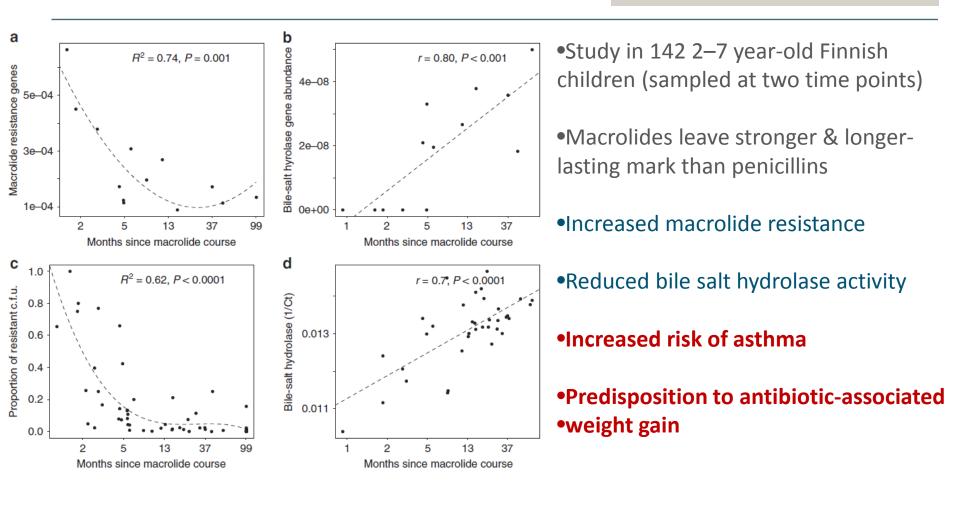


Korpela et al., 2016, Nat.Comm.

•Study in 142 2–7 year-old Finnish children (sampled at two time points)

•Macrolides leave stronger & longerlasting mark than penicillins

Antibiotic use affects microbiota composition & function in pre-school children



Effect of antibiotics

Assess impact of AB treatment of sows on offspring colonization

AB's used for controlling infections of genitourinary tract and mammary gland peri- or post-partum in sows



AB's as model for aberrant microbiota



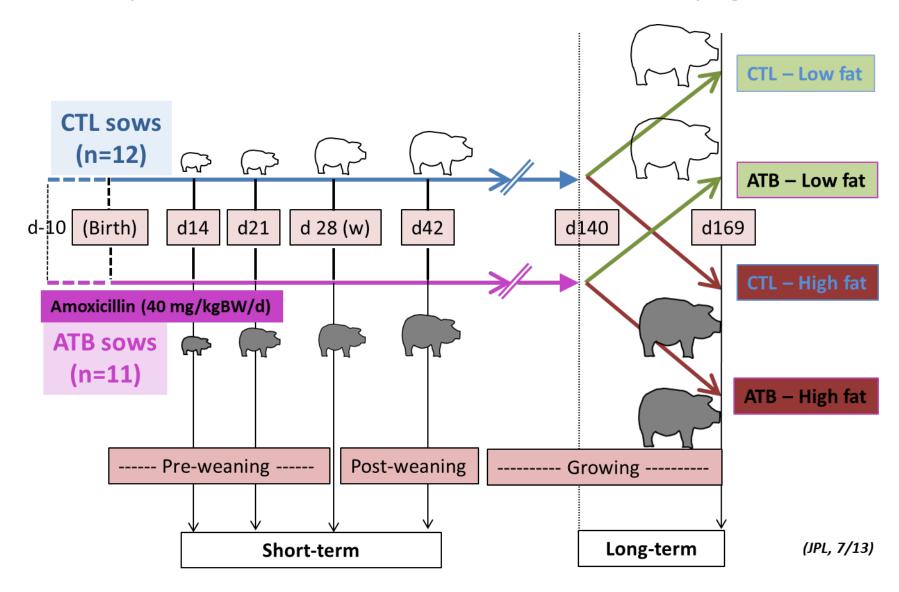




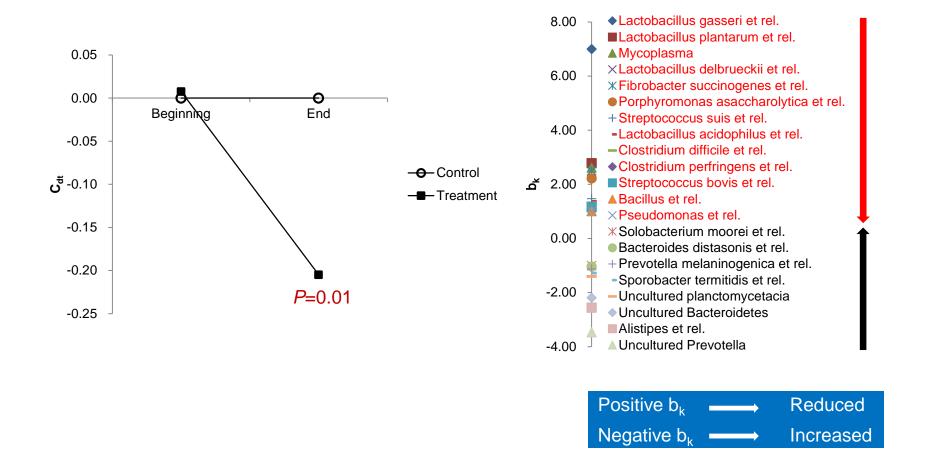
Microbiology



Protocol for short-term and long-term effects of perinatal antibiotic treatment in pigs

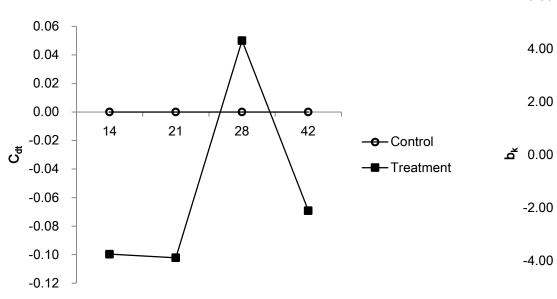


Antibiotic affected microbiota of sows

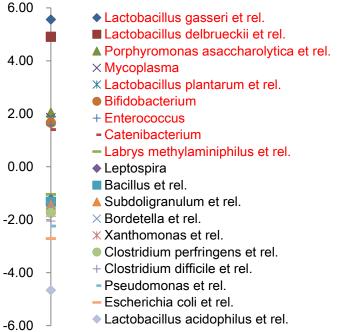


Arnal, Zhang et al., 2014, PlosOne

Maternal antibiotic affects ileal microbiota in offspring



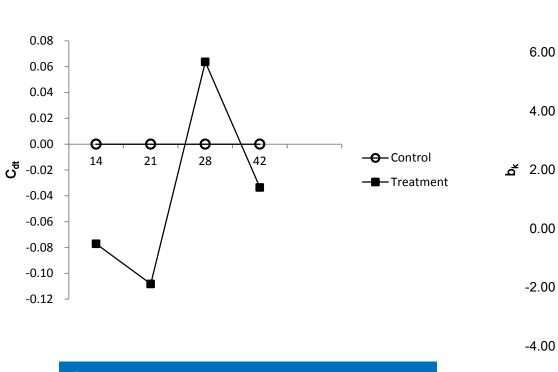
Gut microbiota altered differently after stopping maternal antibiotic administration and after weaning

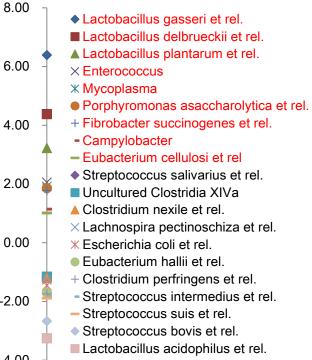


Positive b_k: decreased from day 14 to 21, increased during day 21 and 28, decreased after day 28

Negative b_k: opposite trend

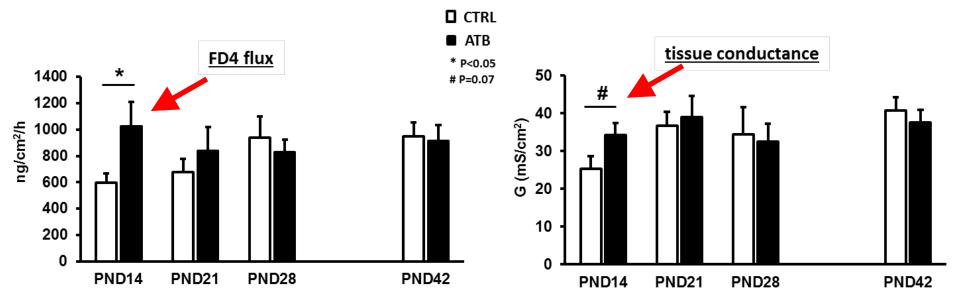
Maternal antibiotic affects colonic microbiota in offspring



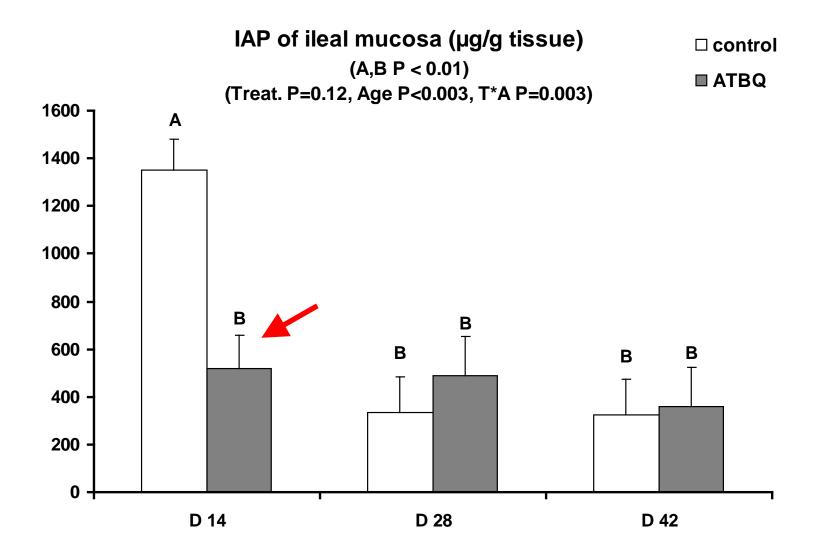


Same trend as ileum

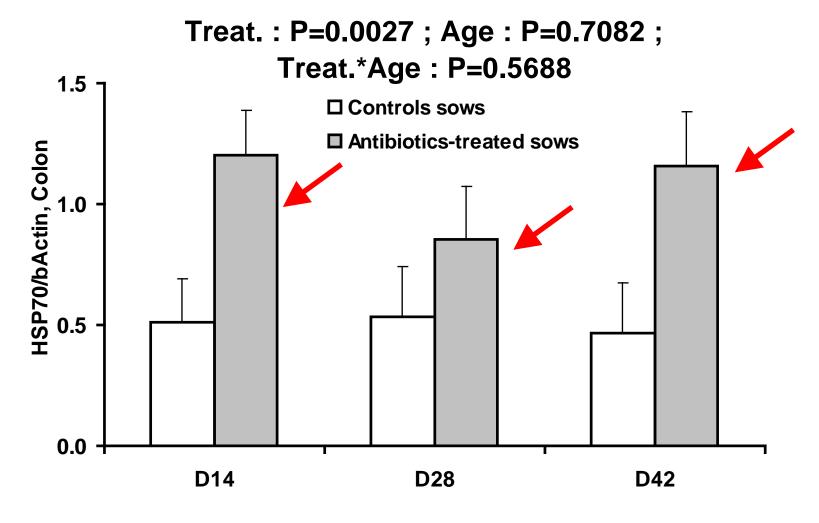
Ileal paracellular permeability is transiently increased in offspring born to antibiotic-treated sows



Ileal IAP is transiently decreased in offspring born to antibiotic-treated sows

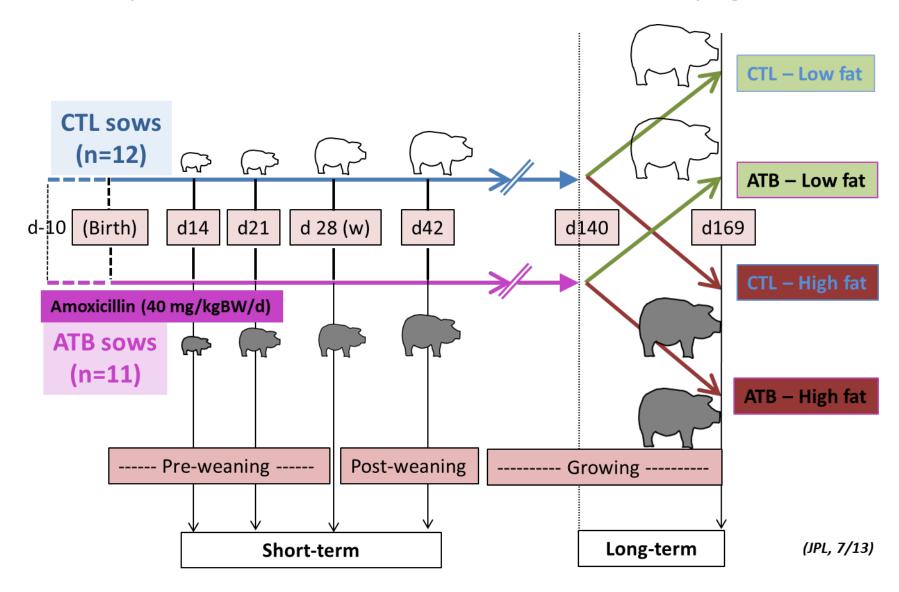


Colonic HSP70 is increased in offspring born to antibiotic-treated sows



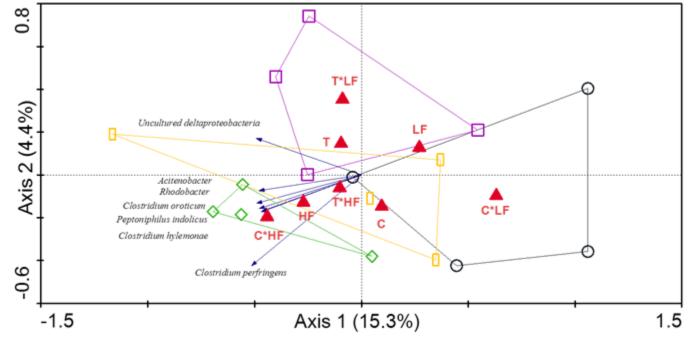
Same results for inducible HSP27

Protocol for short-term and long-term effects of perinatal antibiotic treatment in pigs



Maternal antibiotic treatment has little effects on ileal and colonic microbiota in adult offspring

 \rightarrow Tendency for an interaction between ATB treatment and offspring adult diet (P=0.065):



 \rightarrow No effects on colonic microbiota

Summary of long-term effects

Variable	Site	Perinatal antibiotics	Adult diet (HF)	ATBQ * AD interaction
Villus-crypt architecture	J,I	no	no	no
Alkaline phosphatase	J,I	YES	no	YES
Aminopeptidase N	J,I	no	no	no (# J)
Dipeptidyl-peptidase IV	J,I	YES	no	no
Sucrase	J,I	no	YES	YES
HSP 27 and HSP 70	I	no	no	no
Paracellular perm.	I,Co	no	no	YES
Paracellular perm/OxS	I,Co	no	no	YES
Transcellular perm.	I,Co	no	no	no
Transcellular perm./OxS	6 Co	YES	no	no
PC/TC perm. Ratio	Со	YES	no	no
Basal Isc current	1	YES	no	no
Na ⁺ -Glucose absorpt.	1	no	no	YES
Carbachol- Cl secretion	I	no	no	no

OxS: oxidative stress (monochloramine)

Conclusions

- significant differences in microbiota composition in piglets especially before and after weaning
- effect of AB treatment in sows amplified in piglets AFTER weaning
 - Microbial groups adapted to post-weaning (adult) diet
- Minor long term effects on microbiota composition
- BUT: long-lasting SELECTIVE and REGIONAL effects on key functions of offspring gut

The Team

WAGENINGEN UNIVERSITY & RESEARCH

MICROBIOLOGY

- Odette Perez
- Jing Zhang
- Xin Gao
- Lingli Zhang



Laboratory of Microbiology

INRA

- Jean-Paul Lallès
- Marie-Edith Arnal
- Gaëlle Boudry
- Isabelle Luron

The way forward?

- Will differences early in life make a difference later in life?
 - Evidence from studies with antibiotics
 - To be studied in CCC-Carbobiotics



- Personalized infant nutrition needed/possible?
 - Customized mixes of HMOs (analogues)?
 - Longitudinal in vitro & in vivo personalized dietary intervention studies needed

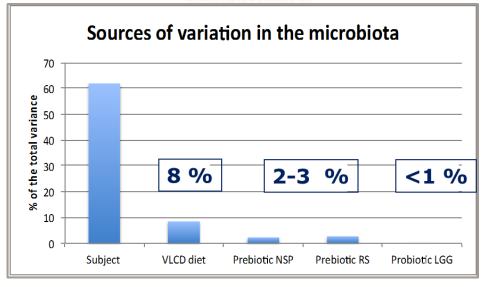
Effect of Diet on Adult Colonic Ecosystem Secondary to Individual Variation

Personalization Overrides Fluctuation Normal Diet



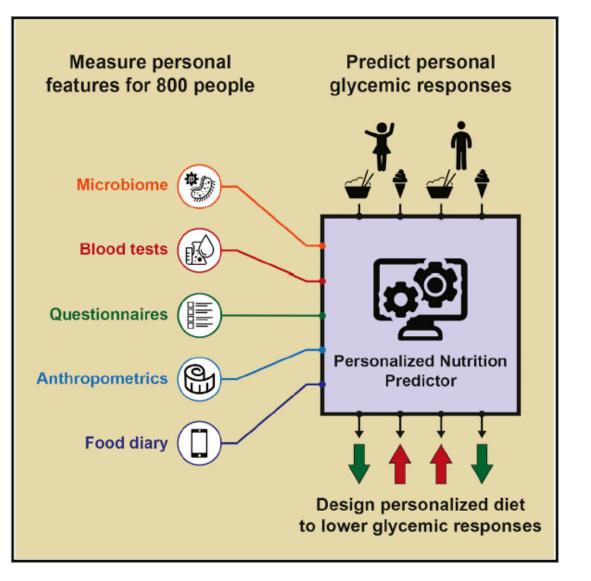
High Temporal Stability Colonic Microbiota – 10 years

The Original Food Pyramid



Salonen et al., 2014

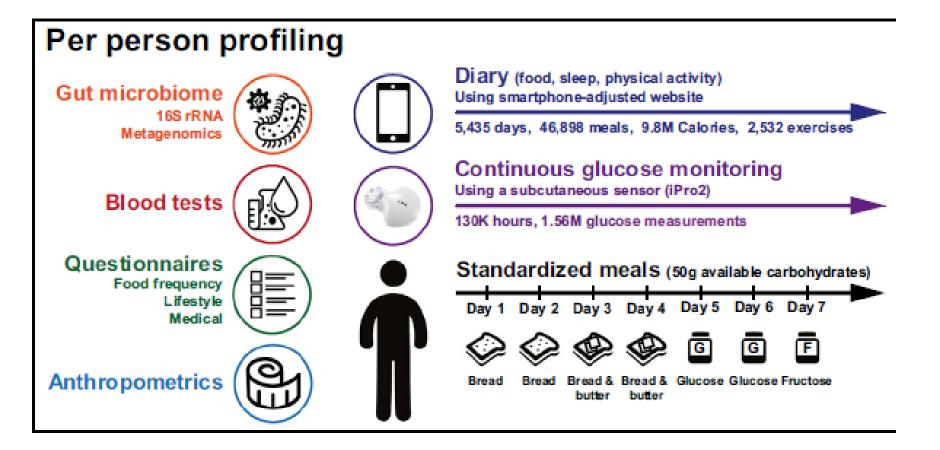
How to personalize nutrition?



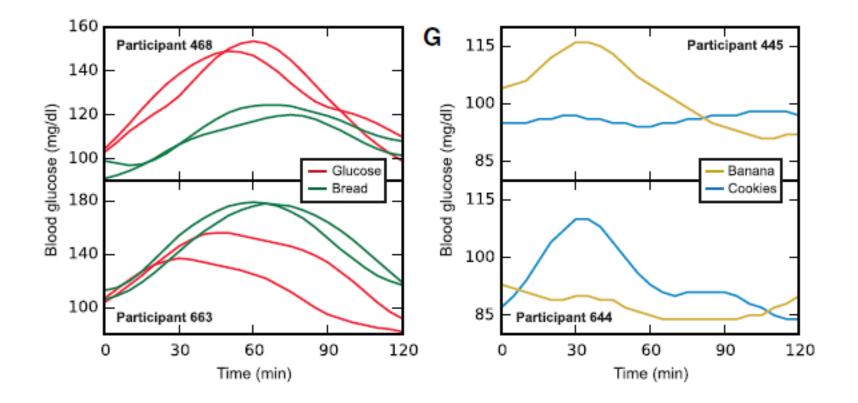
•800 people •46898 meals

Zeevi et al., Cell, 2015

How to personalize nutrition?

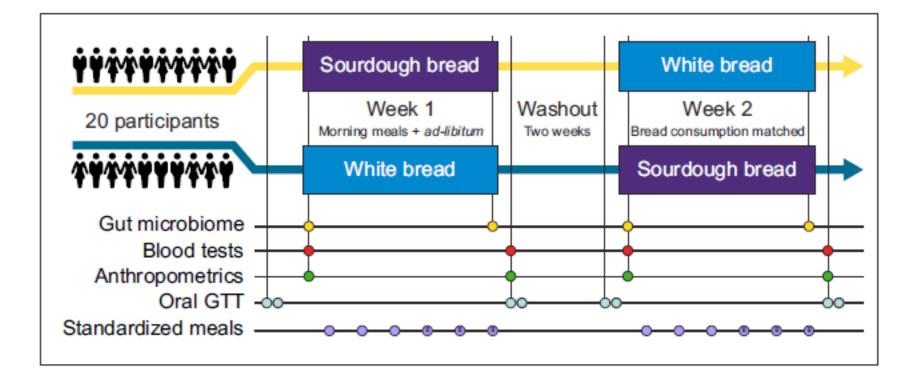


Individuals differ in their glycemic response

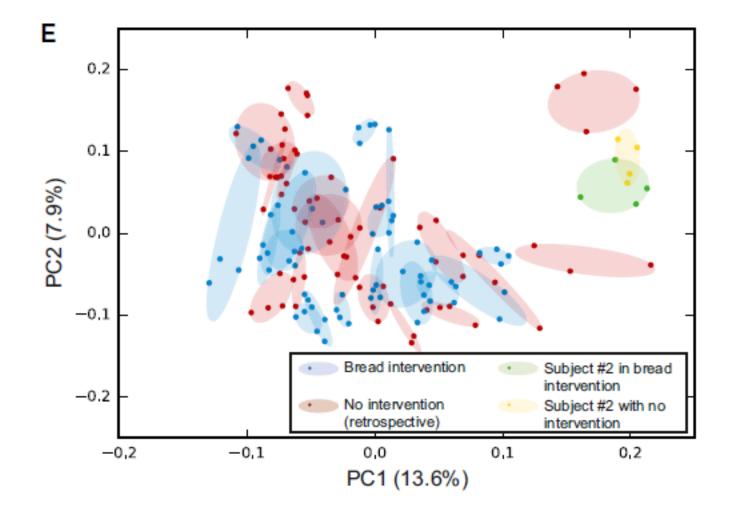


Zeevi et al., Cell, 2015

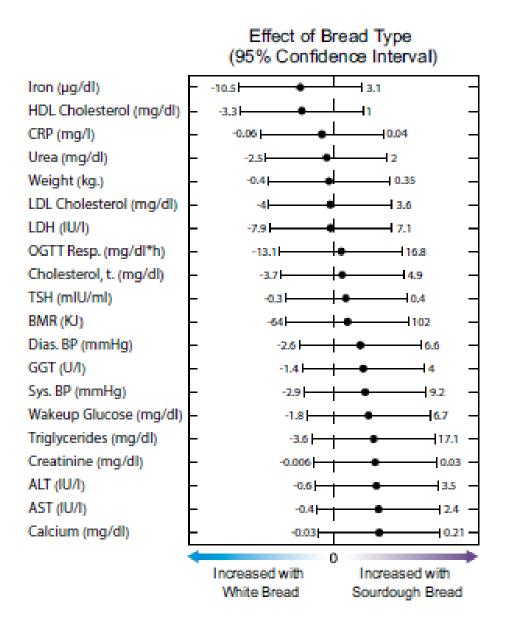
Follow up cross-over study



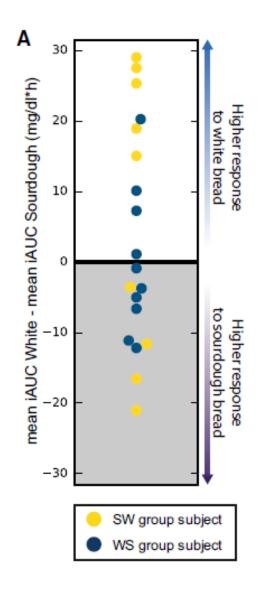
Individual microbiomes remain stable



Bread didn't affect clinical symptoms

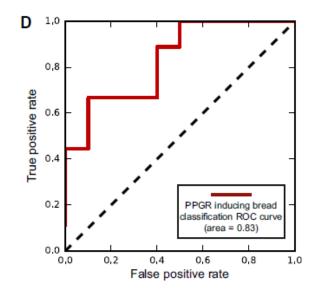


Microbiome predictive of glycemic response



glycemic response to the two types of bread varies greatly across people

Microbiome-based classifier accurately predicts glycemic response-inducing bread type



The Microbiota Revolution



Renaissance of the Intestinal Microbiota Diversity, Enterotypes, Signatures – Functions Early Biomarkers, Network Insight & Novel Therapies

Focus on Microbial Functions

Systems Approaches Developing

Towards a Virtual Gut





Thanks to

All Partners

Study Participants

Microbes



&

YOU