# Mikrobiom und Ernährung

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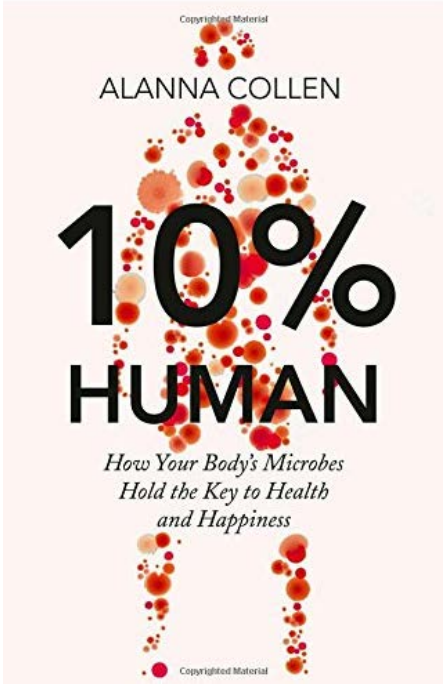
18.09.2017

Christine Kühne – Center for Allergy Research and Education

# Gemeinsamkeiten?




**Zellen:**
- 10% Mensch
- 90% Bakterien

**Gene:**
- 1% Mensch
- 99% Bakterien

# Everything that we eat, drink, touch, and breathe is reflected in our microbiota

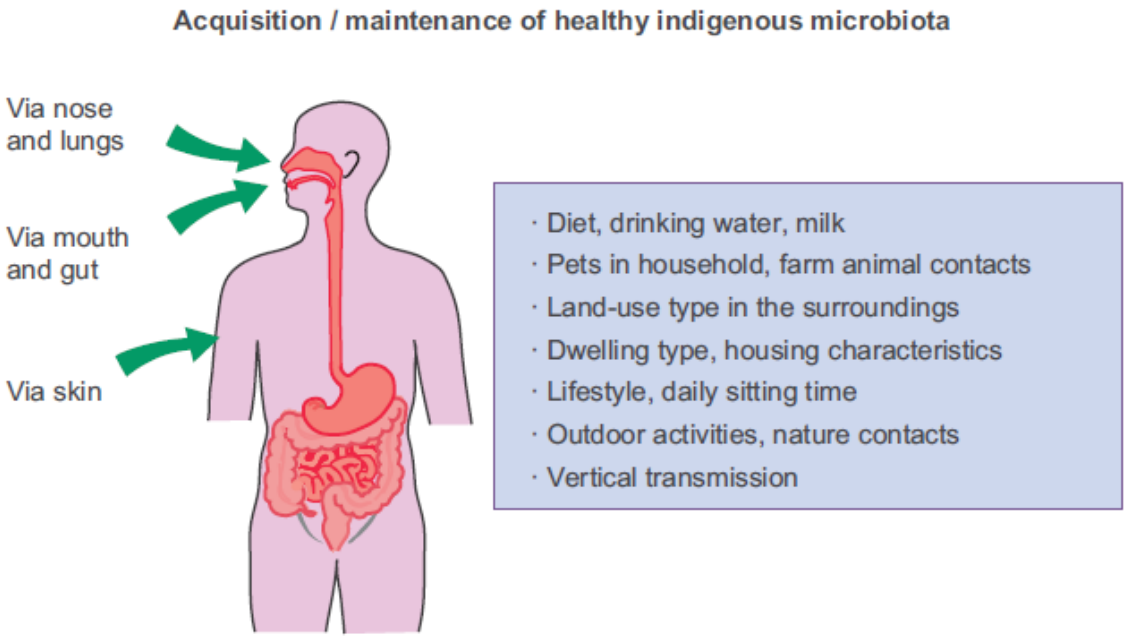



Figure 2. 'We are what we host, and we host what we eat, touch, and breathe'. Outdoor activities in biodiverse environments together with unprocessed food can provide us with microbial exposures necessary for the development and maintenance of healthy balanced microbiota and immunoregulatory circuits.

**Outdoor activities in biodiverse environments together with unprocessed food can provide us with microbial exposures necessary for the development and maintenance of healthy balanced microbiota and immunoregulation and tolerance.**

**Helsinki alert of biodiversity and health**
Von Hertzen L., et al. Annals of Medicine 2015

# Compostion of gut microbiota

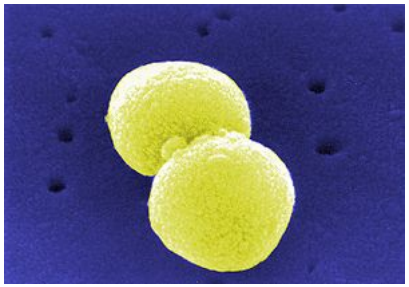
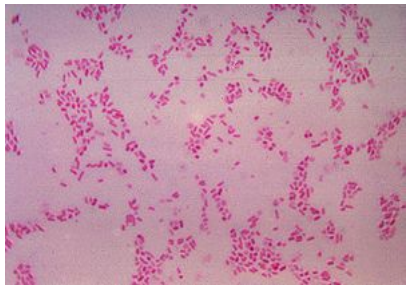
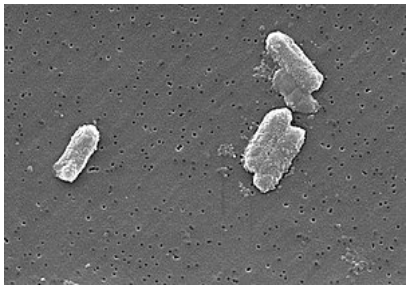
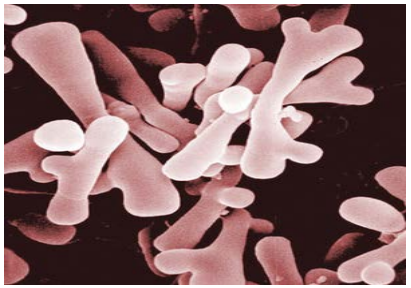
Gut flora to 99% out of 4 phyla:

-Firmicutes: e.g. Lactobacillus, Clostridum

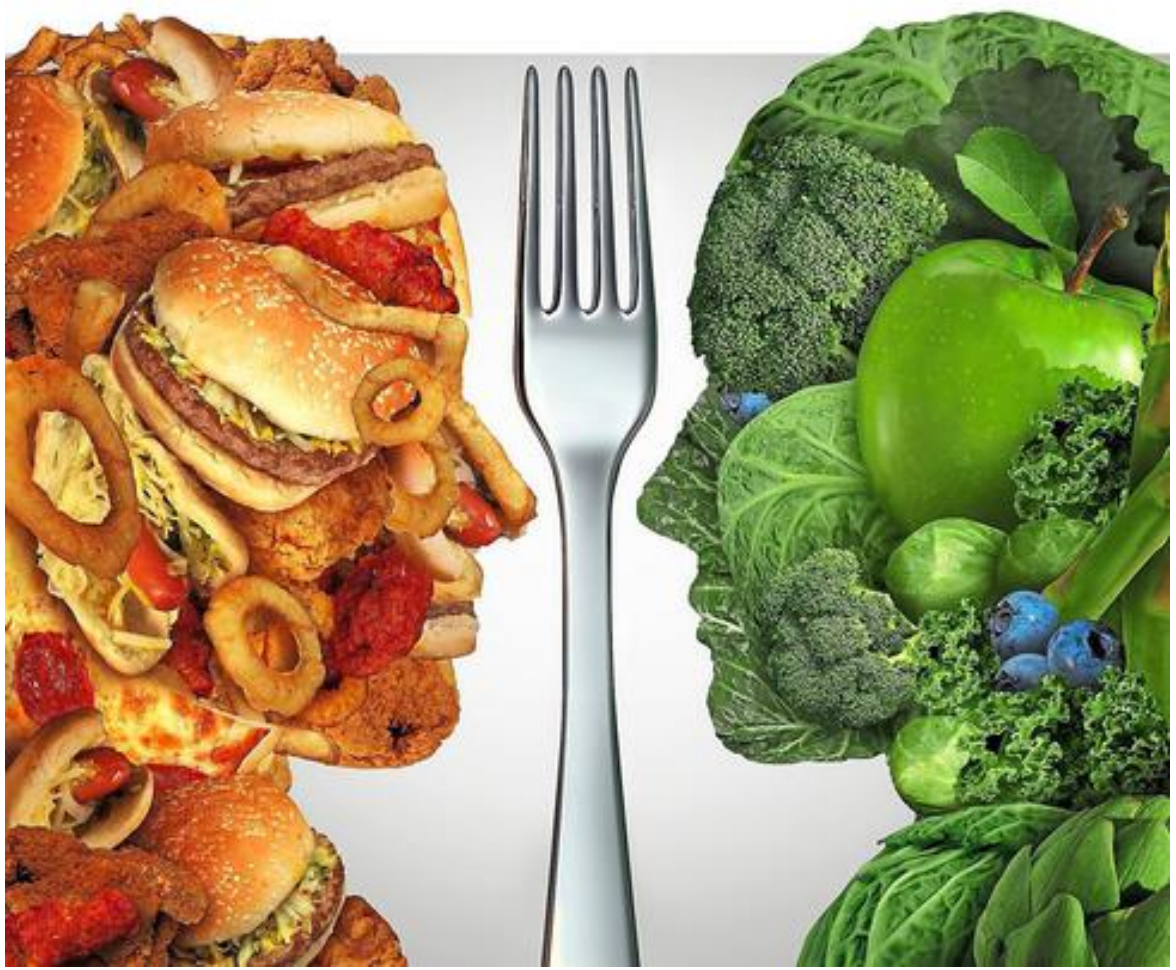
-Bacteroides: $10^{11}$ cells/g -> most common in human gut

-Proteobacteria: e.g. Escherichia

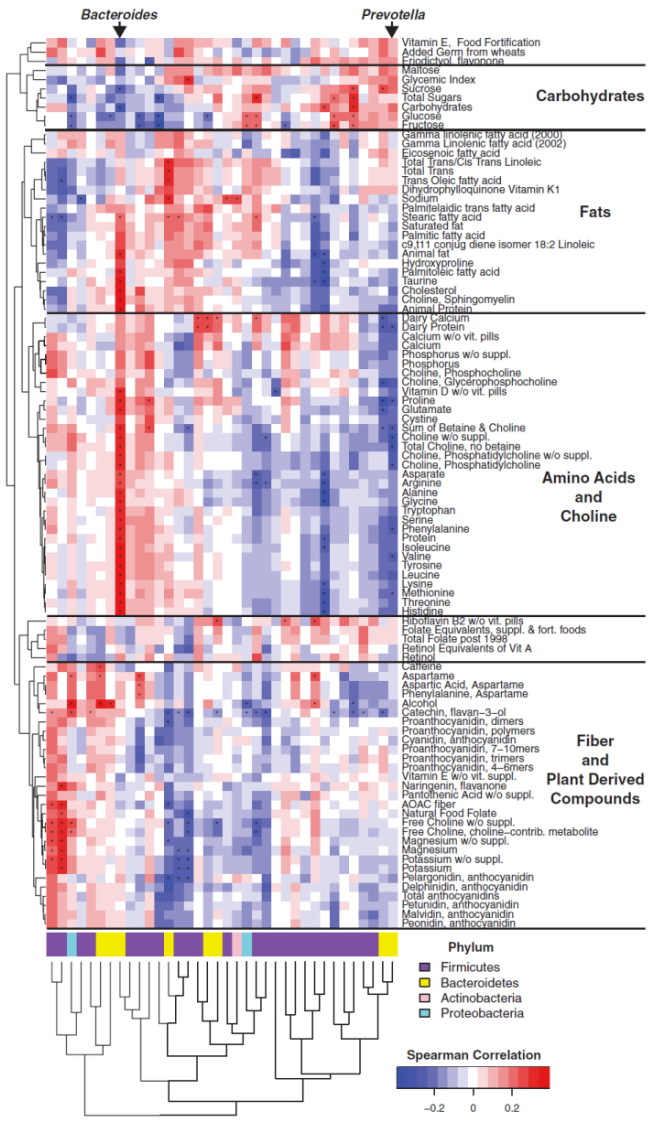
-Actinobacteria: e.g. Bifidobacteria

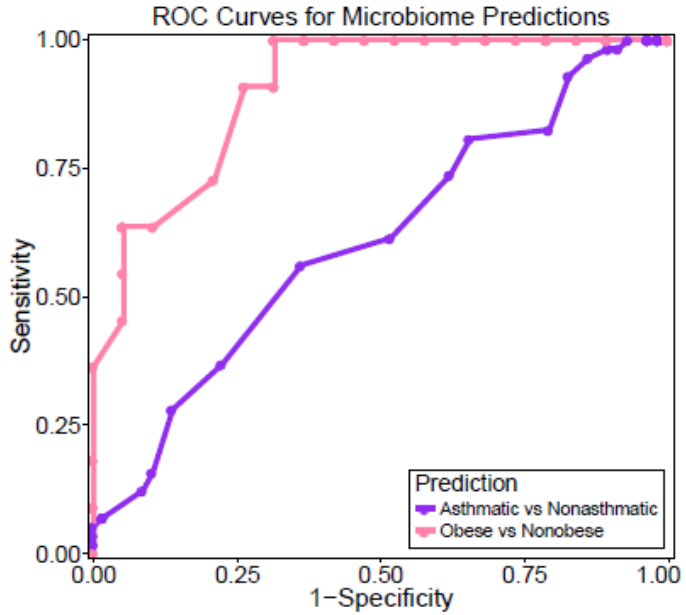
# Is our diet influencing our microbiome?

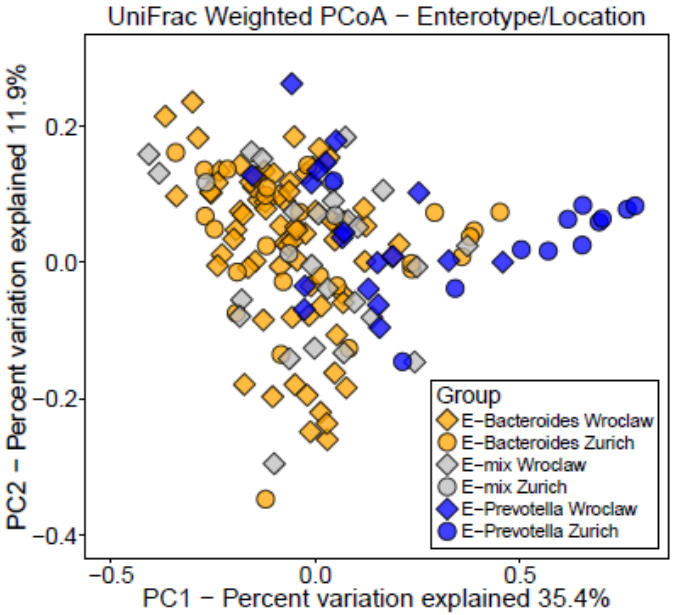


# Diet can influence microbiome



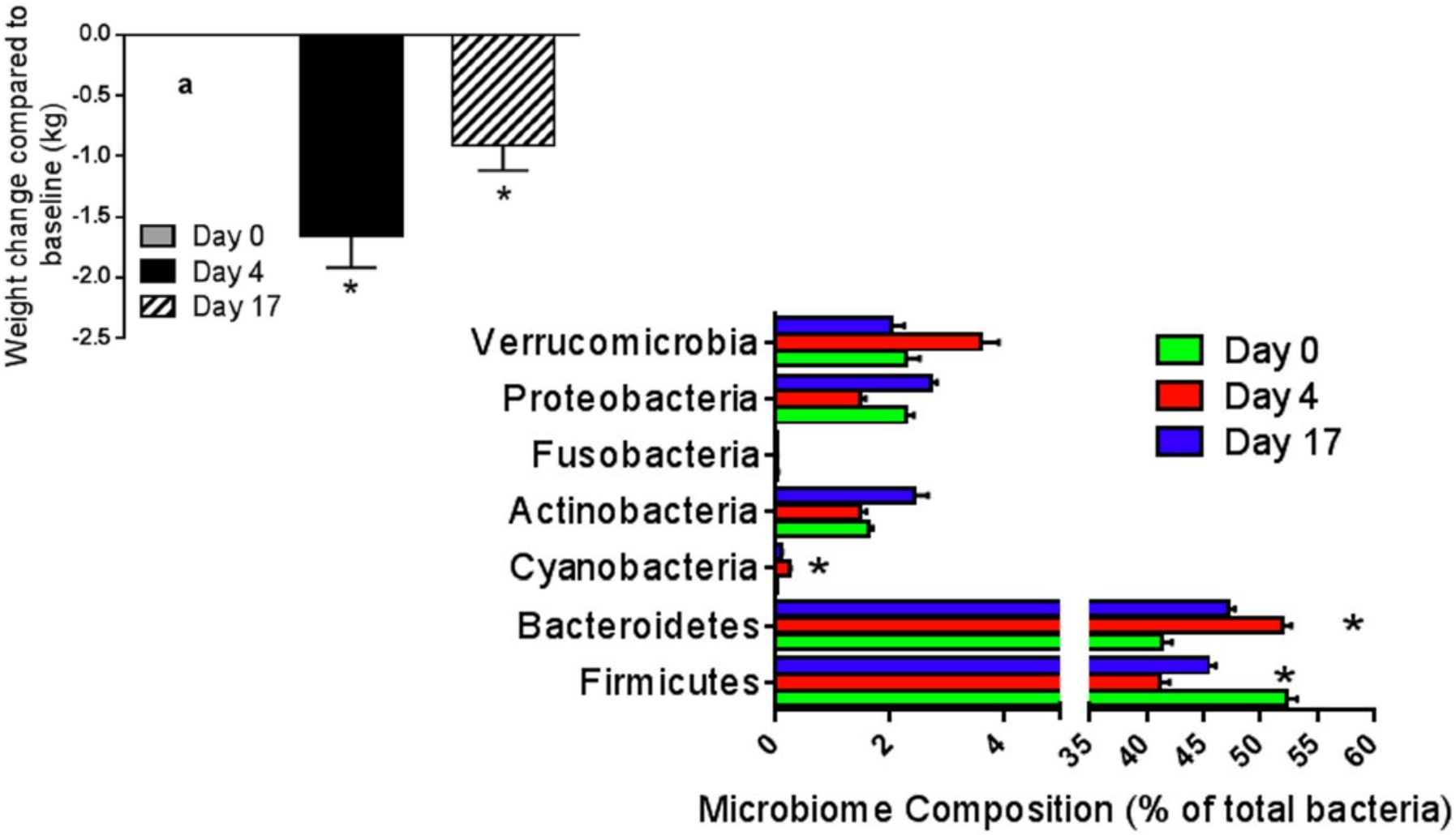

Wu GD, et al., Science 2011




*Manuscript in preperation*

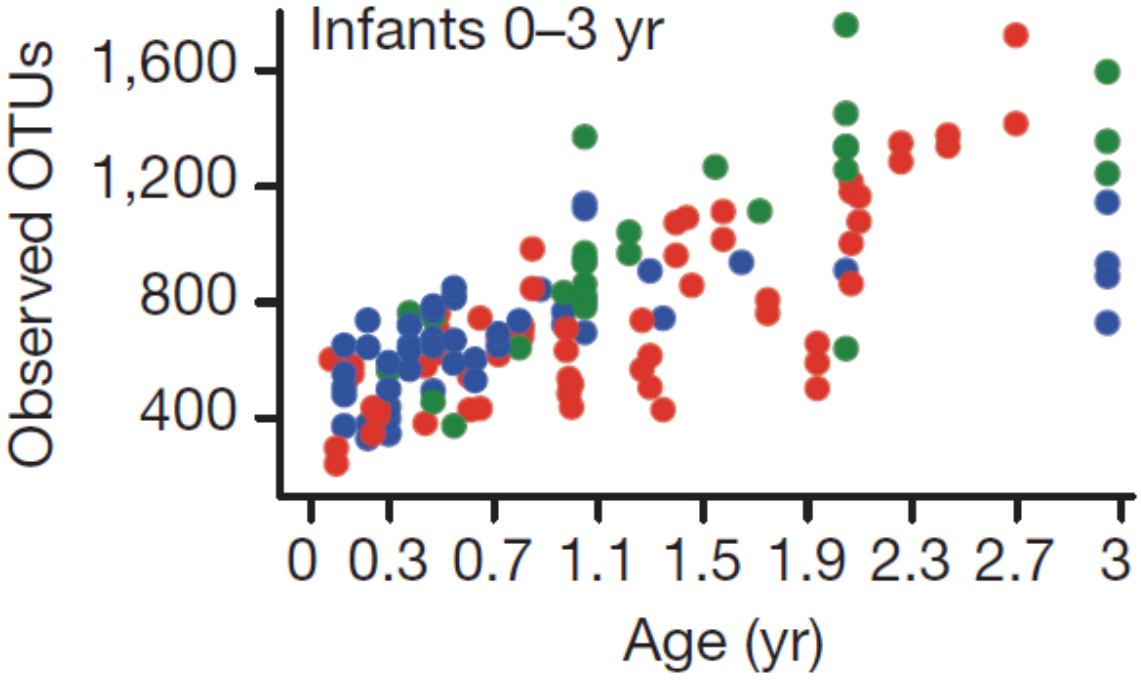
# Influence of a 3 day juice diet

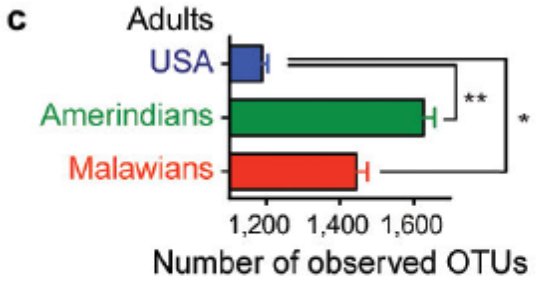



Henning et al., 2017

# When do we start to build up our microbiome?

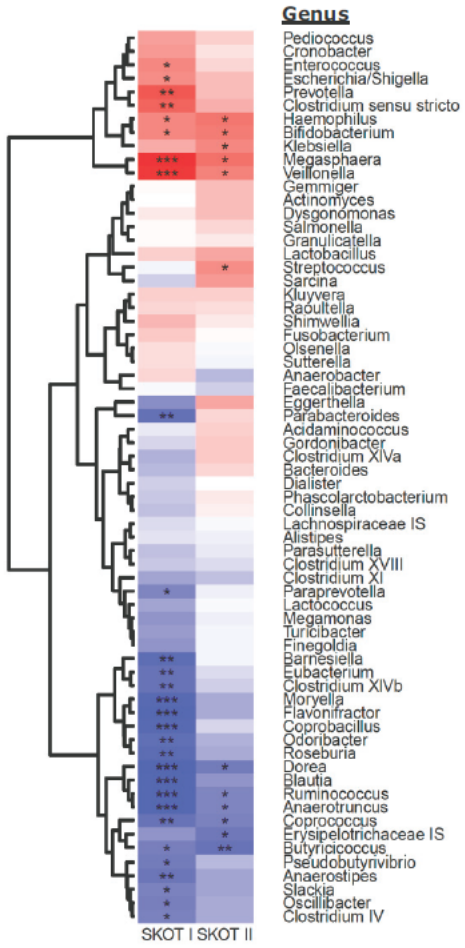

# Establishment of the gut microbiome in childhood




Yatsunenko T. et al. Nature 2012

# Breast-fed versus Formula




| **Firmicutes** | **9 months** | **18 months** | **36 months** |
|---|---|---|---|
| Firmicutes (F1b) | 0,566 | 0,182 | 0,4265 |
| *Lactobacillus* spp. (F2) | 0,0001*** | 0,1736 | 0,4136 |
| *L. acidophilus* (F4) | 0,6249 | 0,172 | 0,9128 |
| *C. butyricum* (F5) | 0,8274 | 0,0561 | 0,6691 |
| *C. leptum* group (F6) | 0,0267* | 0,4015 | 0,5951 |
| *C. coccoides* group (F7) | 0,0021** | 0,0097** | 0,8153 |
| *E. hallii* (F8) | 0,0193* | 0,4321 | 0,6329 |
| *Roseburia* spp. (F9) | 0,0316* | 0,4482 | 0,6901 |
| *Enterococcus* spp. (F10) | 0,0731 | 0,0379* | 0,5529 |
| **Bacteroidetes** | | | |
| Bacteroidetes (B1) | 0,016* | 0,243 | 0,2087 |
| *Bacteroides/Prevotella* groups (B2) | 0,0177* | 0,0128* | 0,1326 |
| *Bacteroides* spp. (B3) | 0,126 | 0,3384 | 0,0709 |
| *B. fragilis* group (B4) | 0,0004*** | 0,0984 | 0,8973 |
| *B. vulgatus* (B5) | 0,0345* | 0,0115* | 0,1967 |
| *B. thetaiotaomicron* (B6) | 0,0016** | 0,017* | 0,3561 |
| *B. eggerthii* (B7) | 0,1241 | 0,9103 | 0,8546 |
| *B. distasonis* (B8) | 0,2906 | 0,1402 | 0,8114 |
| *Prevotella* spp. (B9) | 0,3372 | 0,1112 | 0,076 |
| *Alistipes* spp. (B10) | 0,1745 | 0,2494 | 0,3157 |
| **Bifidobacteria** | | | |
| *Bifidobacterium* spp. (A1b) | 0,0002*** | 0,51 | 0,5306 |
| *B. bifidum* (A2) | 0,6315 | 0,4082 | 0,8208 |
| *B. adolescentis* (A3) | 0,8294 | 0,9744 | 0,946 |
| *B. catenulatum* (A4) | 0,4334 | 0,4076 | 0,7047 |
| *B. longum* (A5) | 0,0477* | 0,8546 | 0,2187 |
| *B. breve* (A6) | 0,7623 | 0,467 | 0,6985 |
| **Other bacteria** | | | |
| *Enterobacteriaceae (P1)* | 0,6179 | 0,6436 | 0,0523 |
| *E. coli* (P2) | 0,6053 | 0,555 | 0,1425 |
| *Desulfovibrio* spp. (P3) | 0,0449* | 0,1072 | 0,9721 |
| *A. muciniphila* (V1) | 0,0451* | 0,0863 | 0,811 |
| *M.smithii* (E1) | 0,0753 | 0,3251 | 0,6047 |

Increased in breastfed at 9 months

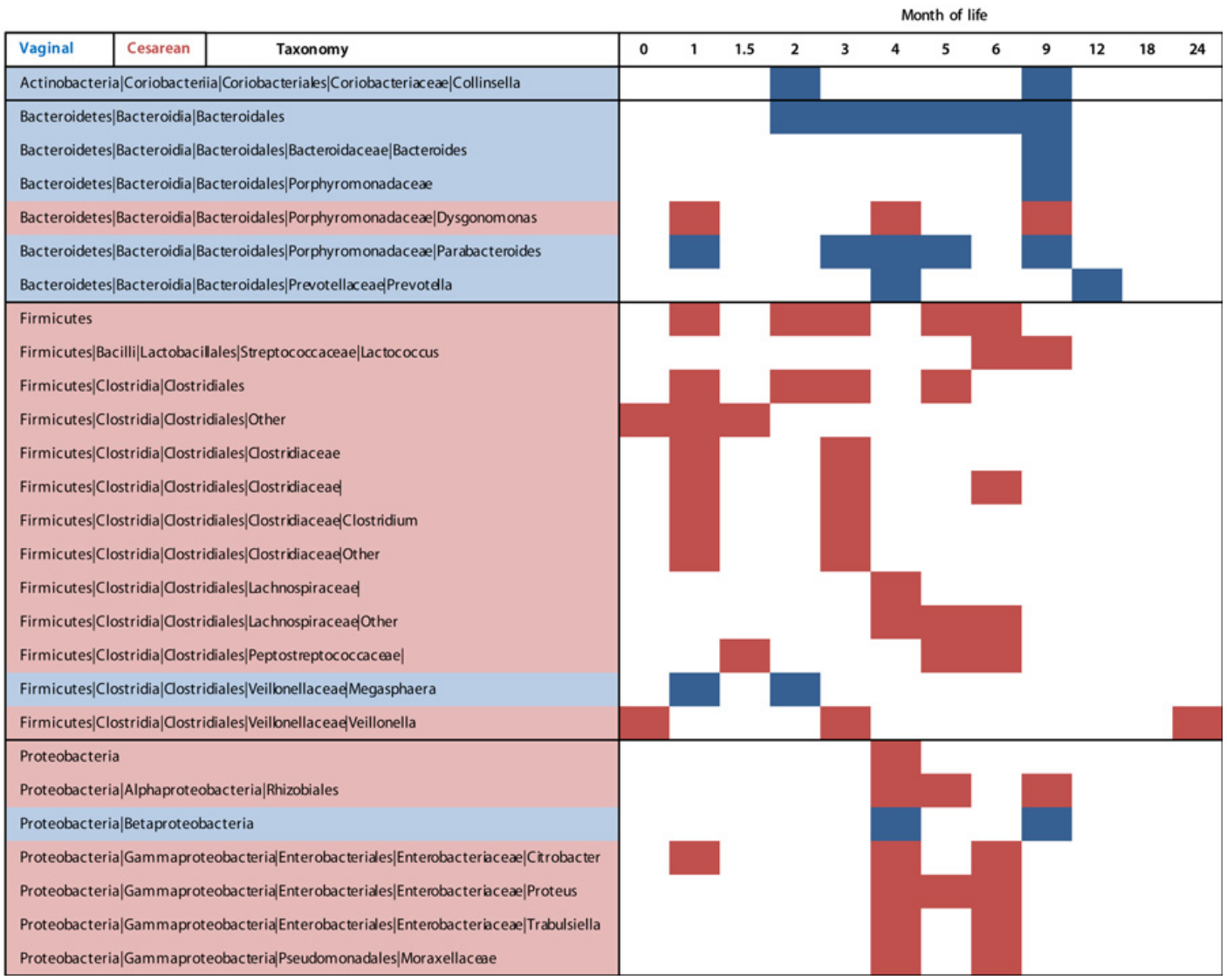
Decreased in breastfed at 9 months

*p<0.05; **p<0.01**; ***p<0.001

Laursen et al, mSphere 2015

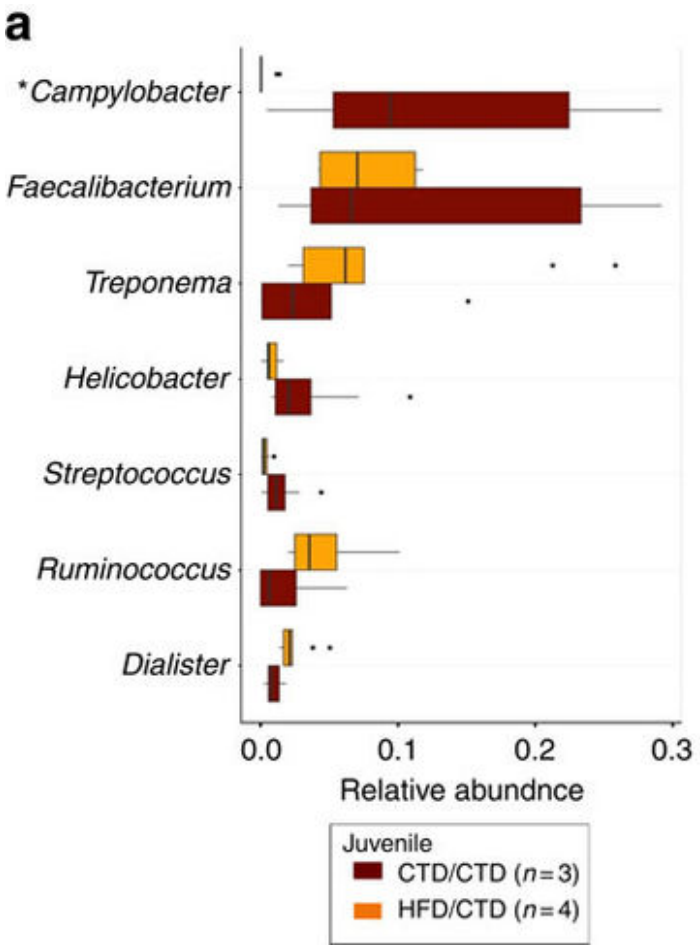
Bergstrom et al, AEM 2014

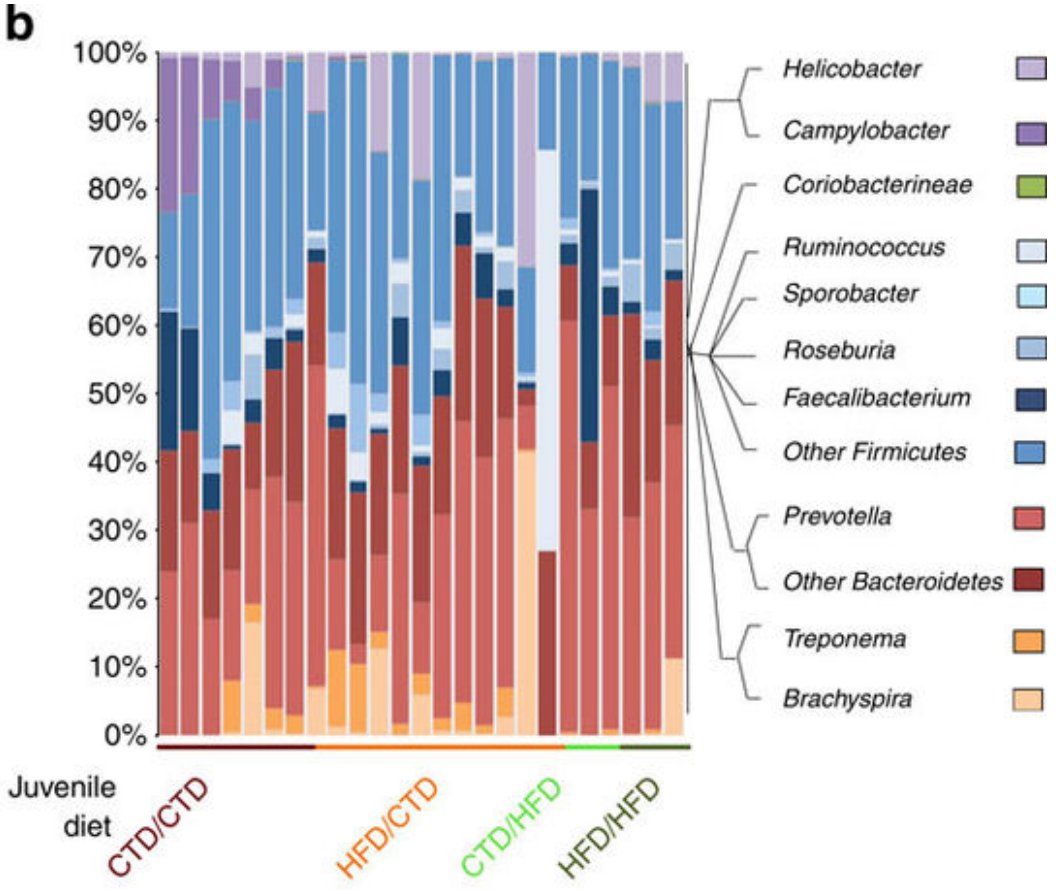
# Vaginal versus C-Section Delivery




Bokulich et al., Sci Transl Med 2016

# Maternal high-fat diet changes microbiome of offspring




Jun et al., Nature Communications 2014

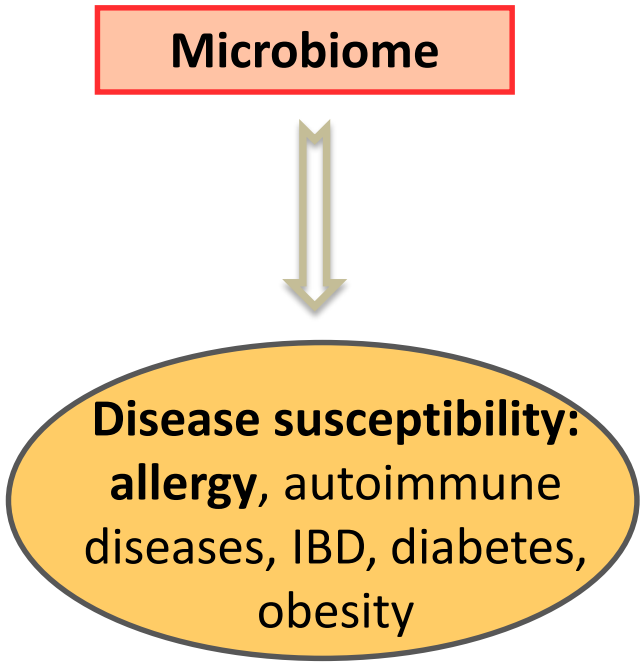
# Environmental and lifestyle factors related to microbial exposure, also related to the risk of developing allergy

**Environment:**

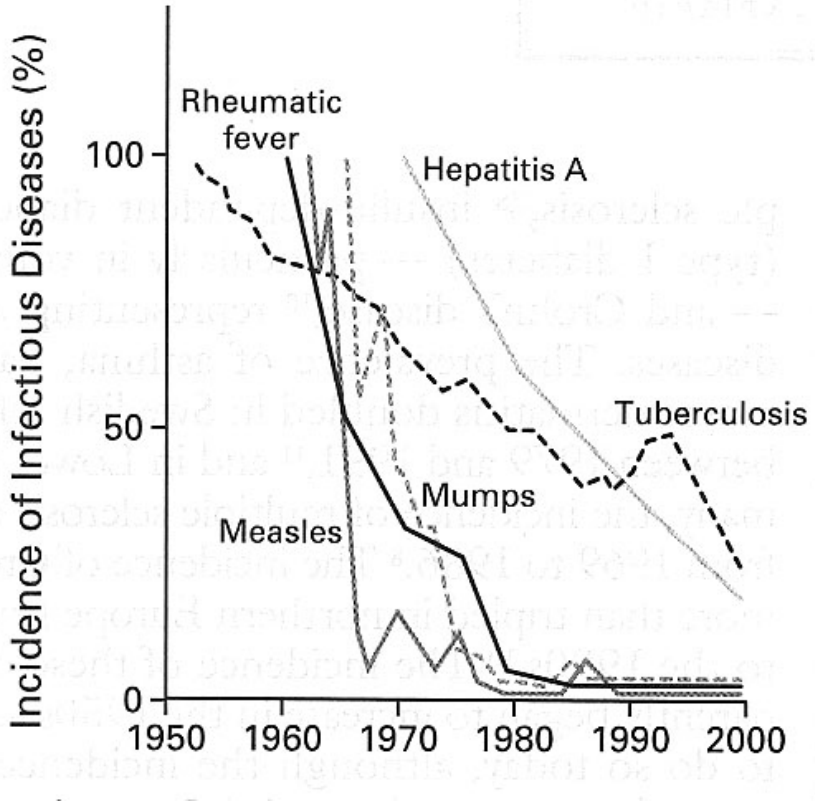
- *Hygiene/ Biodiversity hypothesis*: farming, pet contacts, family size, infections, antibiotic use, mode of delivery

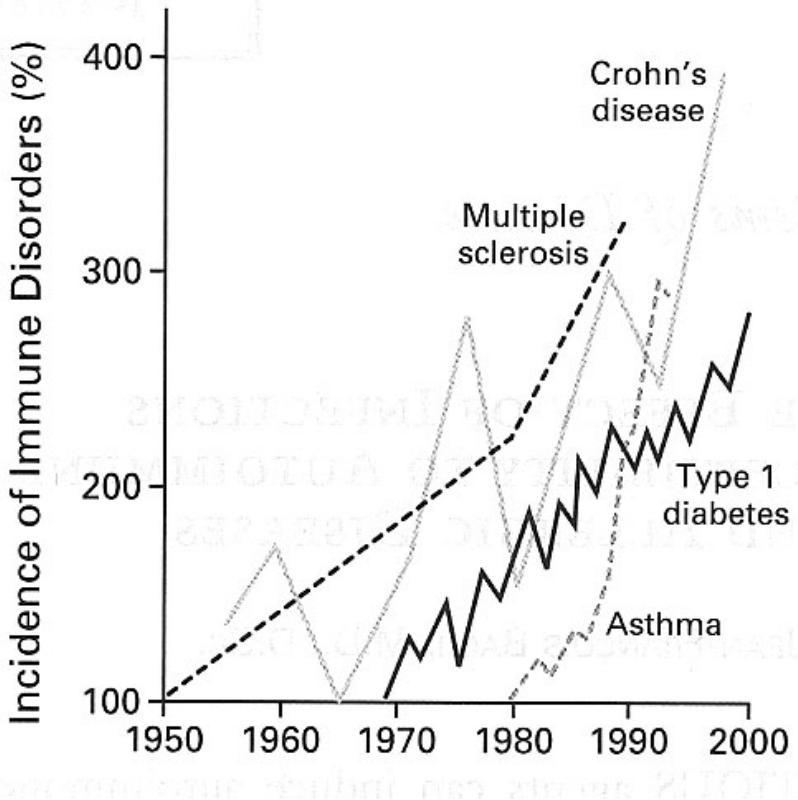
**Diet:**

- *Diet hypothesis*
- *Breastfeeding*



# Infections and Immune-Mediated Disease

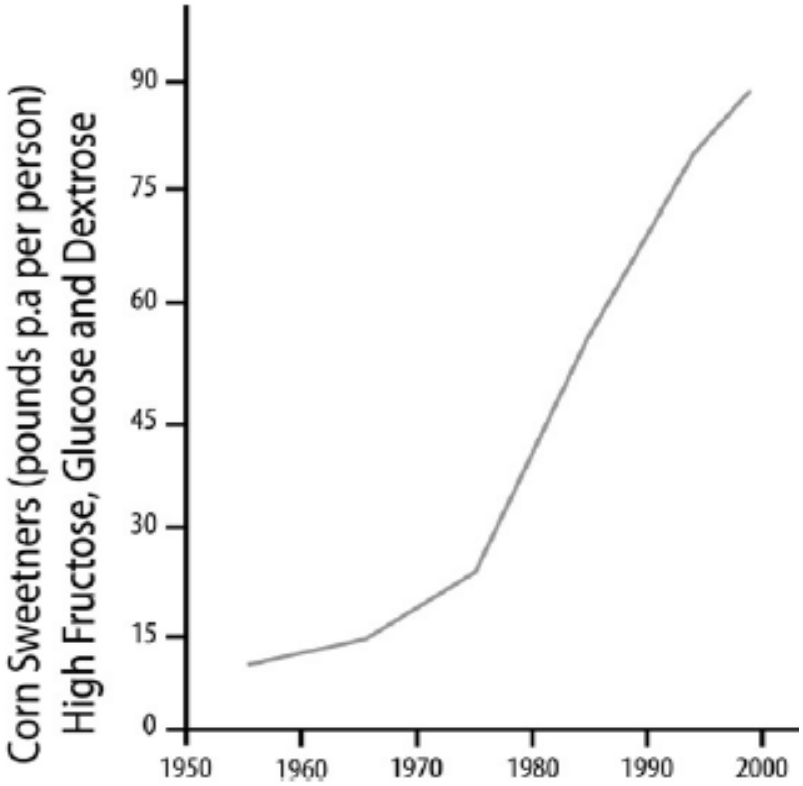


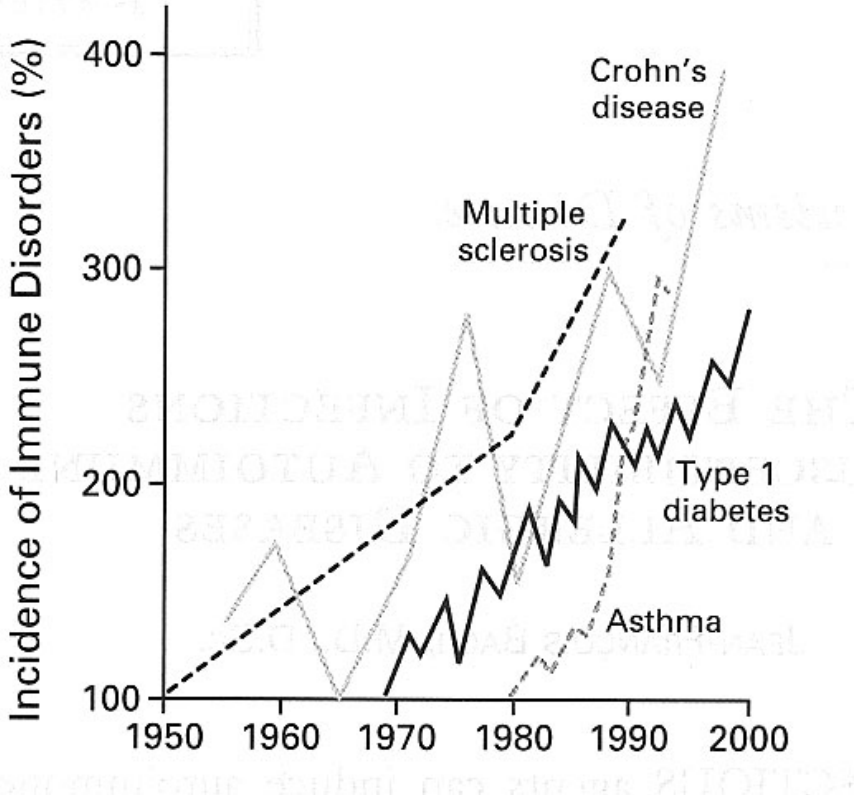

Bach J-F *N Engl J Med* 2002;347:911-20

Exposure to the correct diversity of microbes at the appropriate time

# Diet and Immune-Mediated Disease



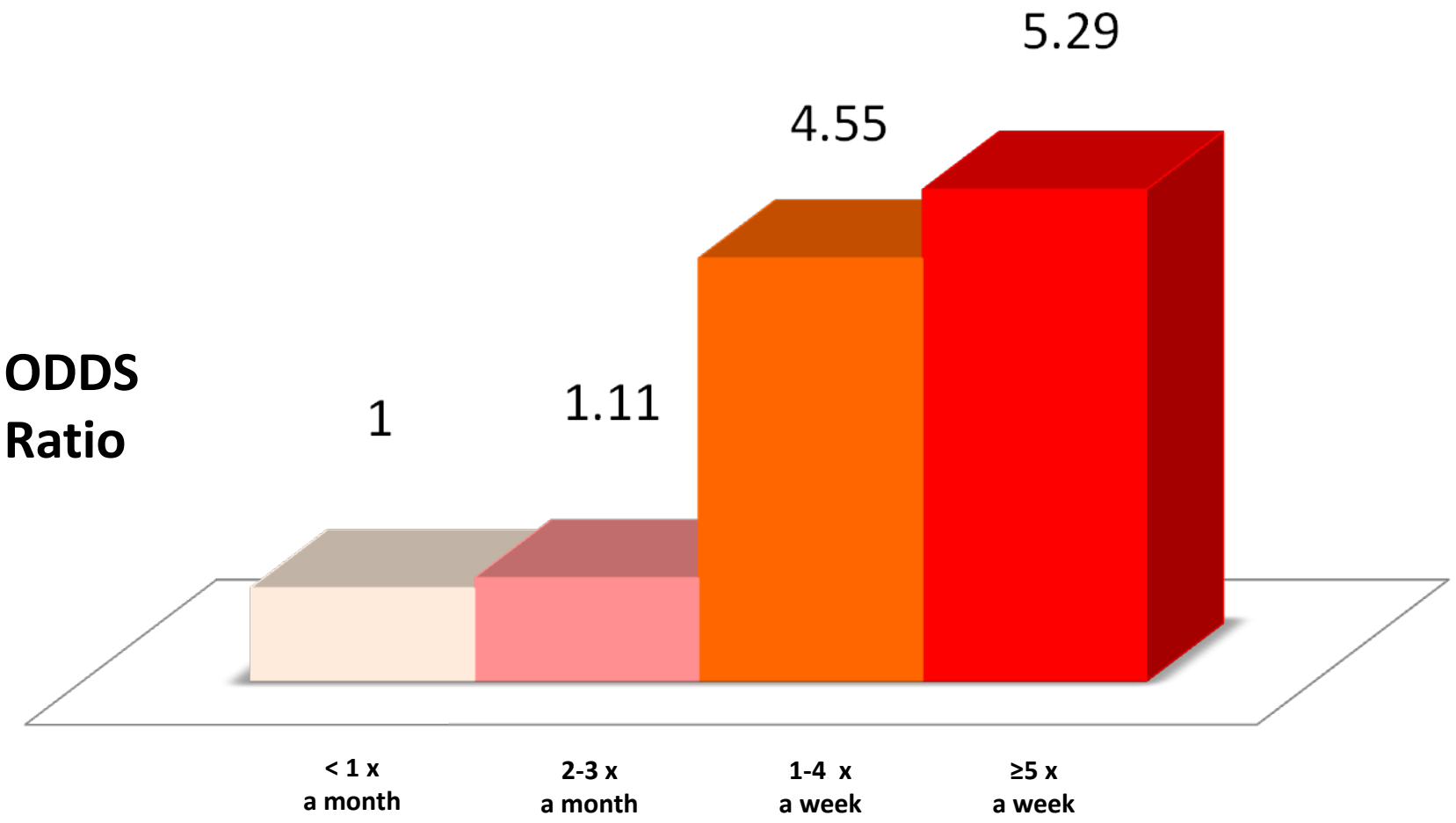

Smith PK *J Allergy Clin Immunol* 2016




Bach J-F *N Engl J Med* 2002;347:911-20

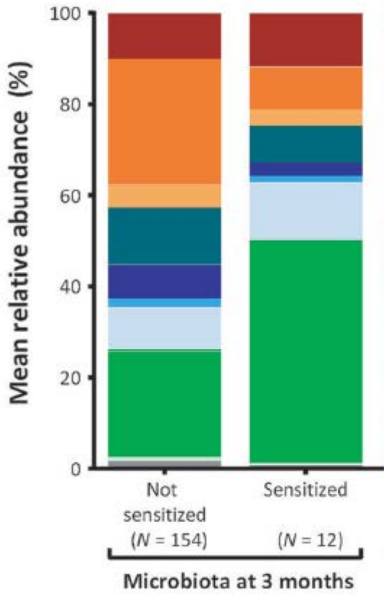
Can diet-microbiota interactions modify immune regulatory mechanisms?

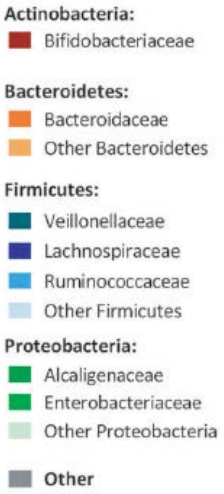
# Total Excess Free Fructose Consumption in 2-9 years

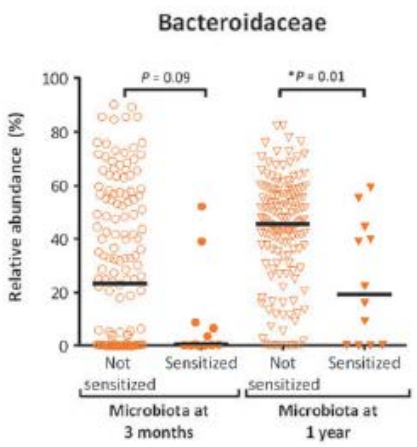


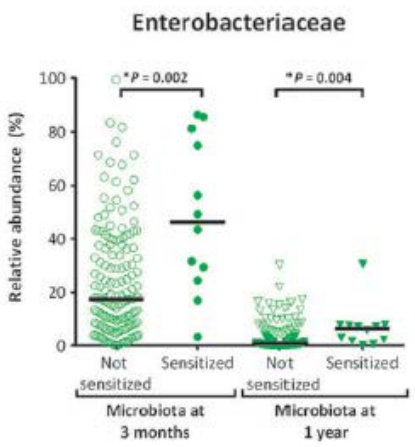

DeChristopher LR, et al. Public Health Nutr. 2016.

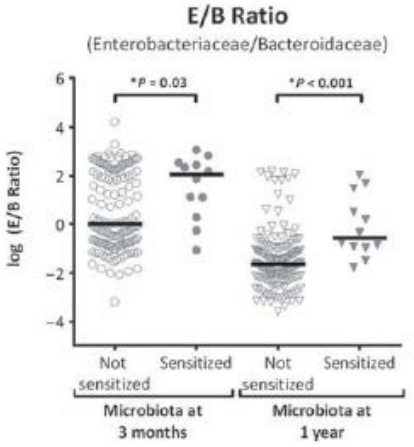
# Microbiota Dysbiosis and Food Allergen Sensitization



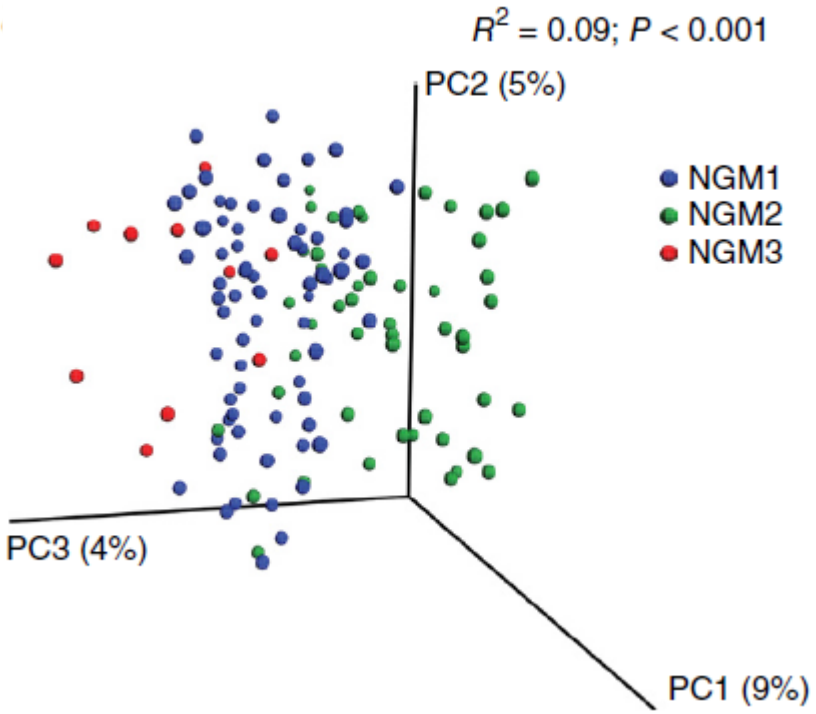



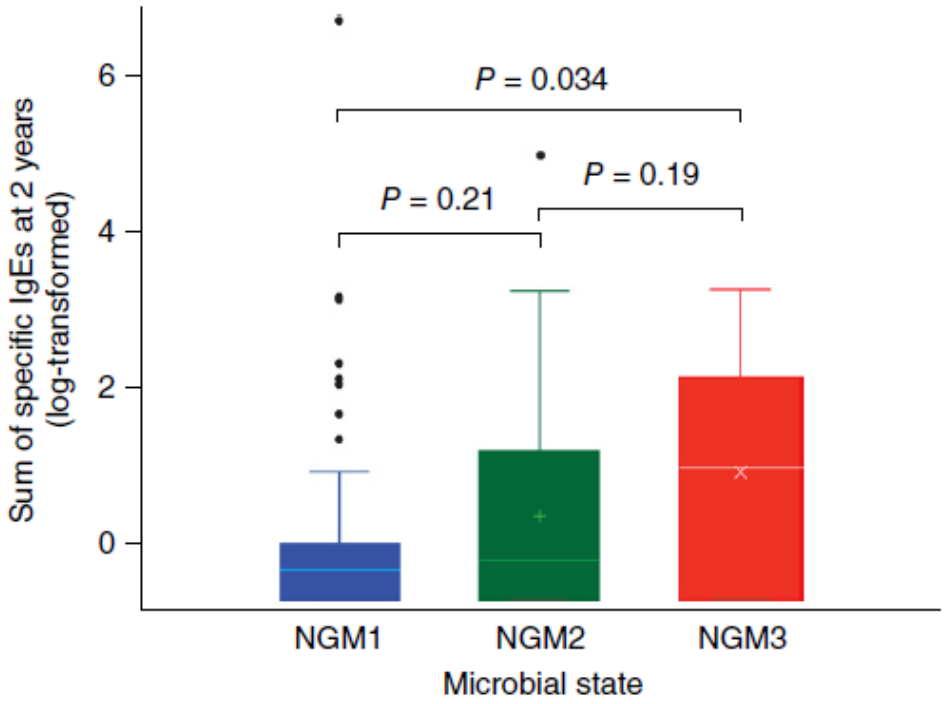




Azad et al., CEA 2015

# Early Life Microbial Dysbiosis Asociated with Atopy Risk




NGM3 has lower relative abundance of Bifidobacteria, Akkermansia and Faecalibacterium

Fujimura et al., Nature Medicine 2016

# A reduced diversity of gut microbiota at age 1 month is predictive for atopic dermatitis

**TABLE II.** Shannon diversity index of the total microbiota, dominant phyla, and significant genera in stool samples obtained at various ages from infants who did or did not have atopic eczema during the first 2 years of life

| | Atopic eczema (n = 20), median | IQR | Healthy (n = 20), median | IQR | *P* value* |
|---|---|---|---|---|---|
| 1 wk | | | | | |
| Total microbiota | 1.59 | 1.33-1.77 | 1.58 | 1.42-1.83 | .78 |
| Firmicutes | 0.81 | 0.48-1.27 | 0.86 | 0.51-1.10 | .53 |
| Proteobacteria | 0.15 | 0.03-0.30 | 0.32 | 0.05-0.37 | .19 |
| Actinobacteria | 0.29 | 0.07-0.41 | 0.27 | 0.10-0.37 | .58 |
| Bacteroidetes | 0.02 | 0.00-0.51 | 0.20 | 0.00-0.39 | .60 |
| 1 mo | | | | | |
| Total microbiota | 1.47 | 1.16-1.66 | 1.69 | 1.53-2.15 | .004 |
| Firmicutes | 0.55 | 0.34-1.11 | 0.61 | 0.44-0.92 | .72 |
| Proteobacteria | 0.15 | 0.06-0.35 | 0.27 | 0.12-0.33 | .29 |
| Actinobacteria | 0.36 | 0.12-0.46 | 0.42 | 0.20-0.67 | .26 |
| Bacteroidetes | 0.05 | 0.00-0.36 | 0.48 | 0.08-0.60 | .02 |
| *Bacteroides* species | 0.01 | 0.00-0.28 | 0.44 | 0.08-0.49 | .01 |
| 12 mo | | | | | |
| Total microbiota | 2.90 | 2.25-3.30 | 2.62 | 2.22-3.27 | .65 |
| Firmicutes | 2.31 | 1.71-2.58 | 1.89 | 1.49-2.39 | .12 |
| Proteobacteria | 0.04 | 0.01-0.07 | 0.07 | 0.04-0.13 | .02 |
| Actinobacteria | 0.21 | 0.11-0.41 | 0.17 | 0.02-0.38 | .43 |
| Bacteroidetes | 0.16 | 0.03-0.36 | 0.50 | 0.12-0.65 | .08 |

*IQR*, Interquartile range.
*Mann-Whitney *U* test.

Abrahamsson et al. JACI 2012

# Antibiotic Use and Food Allergy

**Table 2.** Adjusted* associations of antibiotic orders with milk allergy at different ages of diagnosis up to age 7 years

| | | Age at milk allergy diagnosis** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Diagnosed up to 300 days (0.82 years) | | | Diagnosed after 300 days (0.82 years) | | | Diagnosed up to 7 years | | |
| | Antibiotic order count | Odds ratio | 95% confidence interval | | Odds ratio | 95% confidence interval | | Odds ratio | 95% confidence interval | |
| Milk allergy cases, with or without other allergy diagnoses† | 1-2 vs. none | 1.43 | 0.97 | 2.09 | 1.51 | 1.04 | 2.21 | 1.49 | 1.15 | 1.96 |
| | 3+ vs. none | 2.39 | 1.25 | 4.59 | 1.66 | 1.11 | 2.49 | 1.78 | 1.28 | 2.48 |
| Milk allergy cases excluding cases with another allergy diagnosis‡ | 1-2 vs. none | 1.35 | 0.84 | 2.17 | 0.98 | 0.59 | 1.64 | 1.23 | 0.87 | 1.75 |
| | 3+ vs. none | 3.65 | 1.75 | 7.60 | 1.03 | 0.61 | 1.74 | 1.54 | 1.01 | 2.37 |

* Adjusted for Medical Assistance and race/ethnicity.
†Includes milk allergy cases with and without diagnoses of other allergies: < 301 days includes 279 cases vs. 1395 controls; >300 days includes 205 cases vs. 1025 controls; diagnosed up to 7 years includes 484 cases vs. 2420 controls.
‡Excludes cases with a diagnosis of another allergy: < 301 days includes 200 cases vs. 1000 controls; >300 days includes 114 cases vs. 570 controls; diagnosed up to 7 years includes 314 cases vs. 1570 controls.

Hirsch et al., 2016 CEA

# Probiotics and Food Allergy

*Allergology International.* 2012;61:107-113
DOI: 10.2332/allergolint.11-OA-0305

ORIGINAL ARTICLE

## Prophylactic Probiotics Reduce Cow's Milk Protein Intolerance in Neonates after Small Intestine Surgery and Antibiotic Treatment Presenting Symptoms That Mimics Postoperative Infection

Shoichi Ezaki[1], Kanako Itoh[1], Tetsuya Kunikata[1], Keiji Suzuki[1], Hisanori Sobajima[1] and Masanori Tamura[1]

| | Probiotics (+) $n = 18$ | Probiotics (-) $n = 12$ | $p$-value |
|---|---|---|---|
| Gestational age, weeks | 34.5 (23.5-38.4) | 34.4 (26.4-40.0) | 0.67 |
| Birth weight, g | 1978 (296-3646) | 2004 (542-3522) | 0.82 |
| Age at surgery, days | 4 (0-64) | 1.5 (0-16) | 0.13 |
| Disease requiring surgery | | | |
| Small intestinal atresia | 12 | 10 | 0.42 |
| Localized intestinal perforation | 6 | 2 | |
| Surgical procedure | | | |
| Development of fistulae | 11 | 6 | 0.71 |
| Anastomosis | 7 | 6 | |
| Nutrition | | | |
| Age after surgery when enteral feeding was initiated, days | 7.5 (3-13) | 6.0 (3-10) | 0.06 |
| Type of nutrition | | | |
| Breast milk | 7 | 5 | 1.0 |
| Breast milk and formula or Artificial milk | 11 | 7 | |
| Family history of allergic disease | 2 (11) | 2 (17) | 1.0 |
| Cases of milk intolerance | 1 (6) | 8 (67) | <0.001 |

# Reduced diversity of gut microbiota at age 1 month predictive for asthma

doi: 10.1111/cea.12253

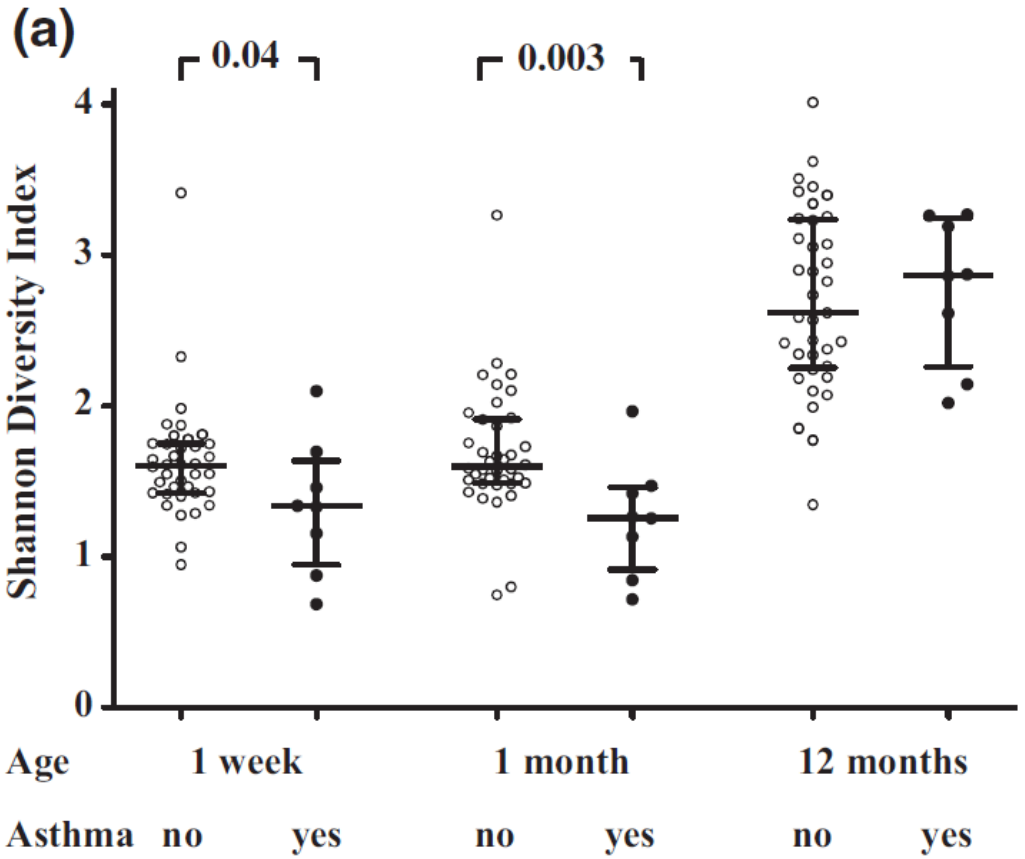
*Clinical & Experimental Allergy*, **44**, 842–850



**ORIGINAL ARTICLE** Clinical Mechanisms in Allergic Disease

## Low gut microbiota diversity in early infancy precedes asthma at school age

T. R. Abrahamsson[1], H. E. Jakobsson[2], A. F. Andersson[3], B. Björkstén[4,5], L. Engstrand[2,3] and M. C. Jenmalm[1,6]



# Gut microbiome and allergy

- A greater abundance of *Bacteroides* and *Lactobacillus* species has been associated with protection against allergy
- Abundance of *Clostridia* species has been positively associated with wheezing, allergic sensitization and atopic dermatitis

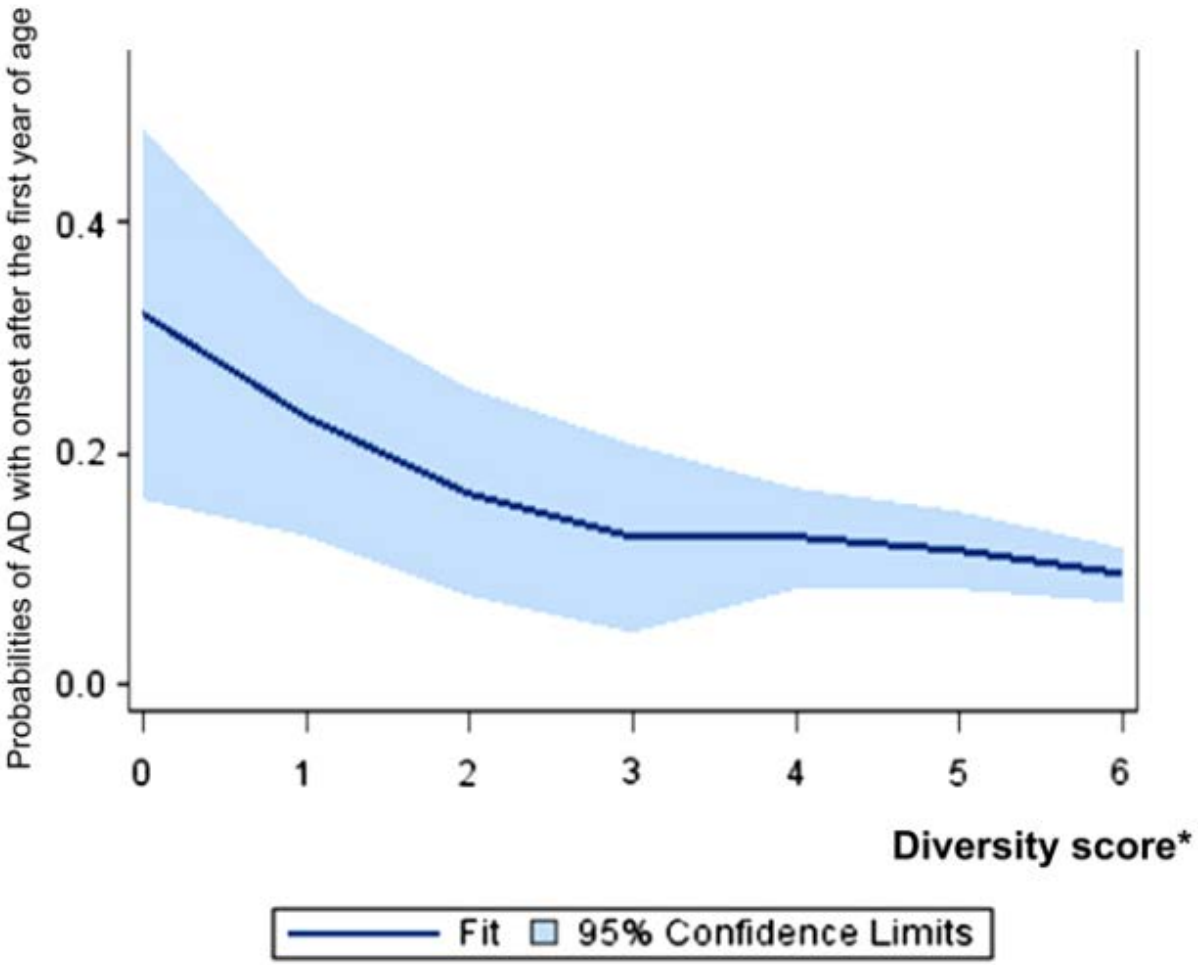

- Van Nimwegen FA et al. JACI 2011
- Penders J et al. JACI 2013

# Diet hypothesis

## Lifestyle changes in diet in westernised countries:

Strong reduction of consumption of **dietary fiber** in westernized countries compared to developing countries

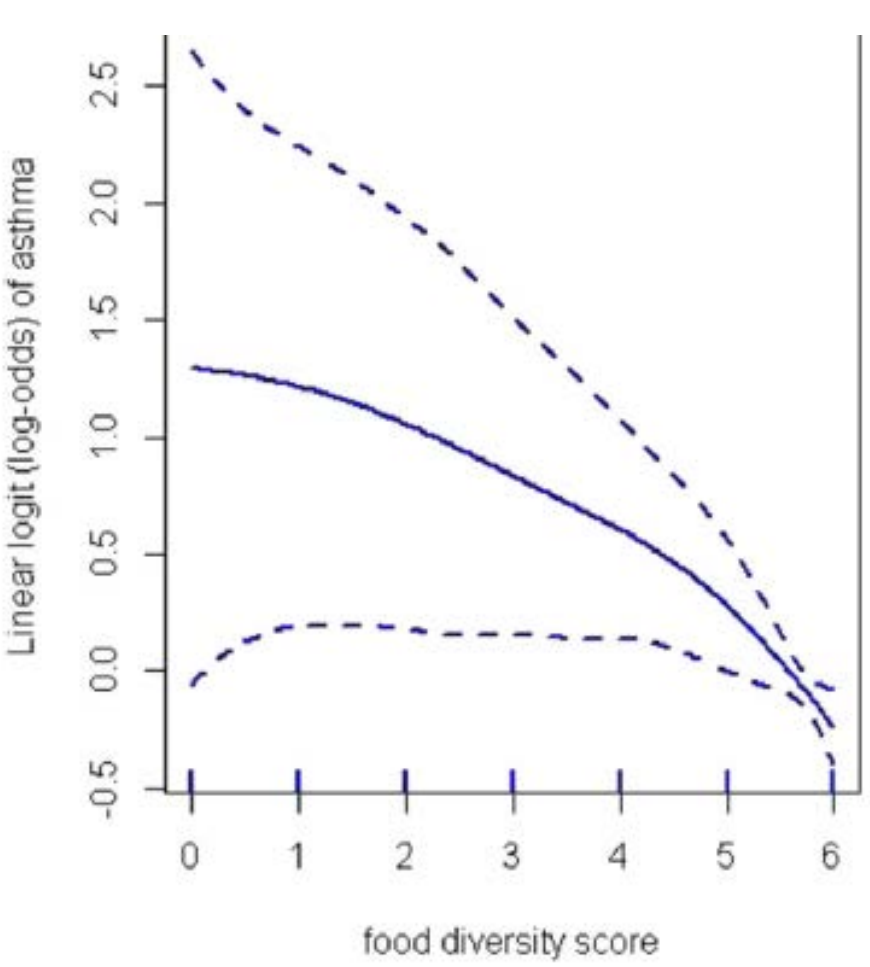
# Food diversity in the 1st year of life and atopic dermatitis

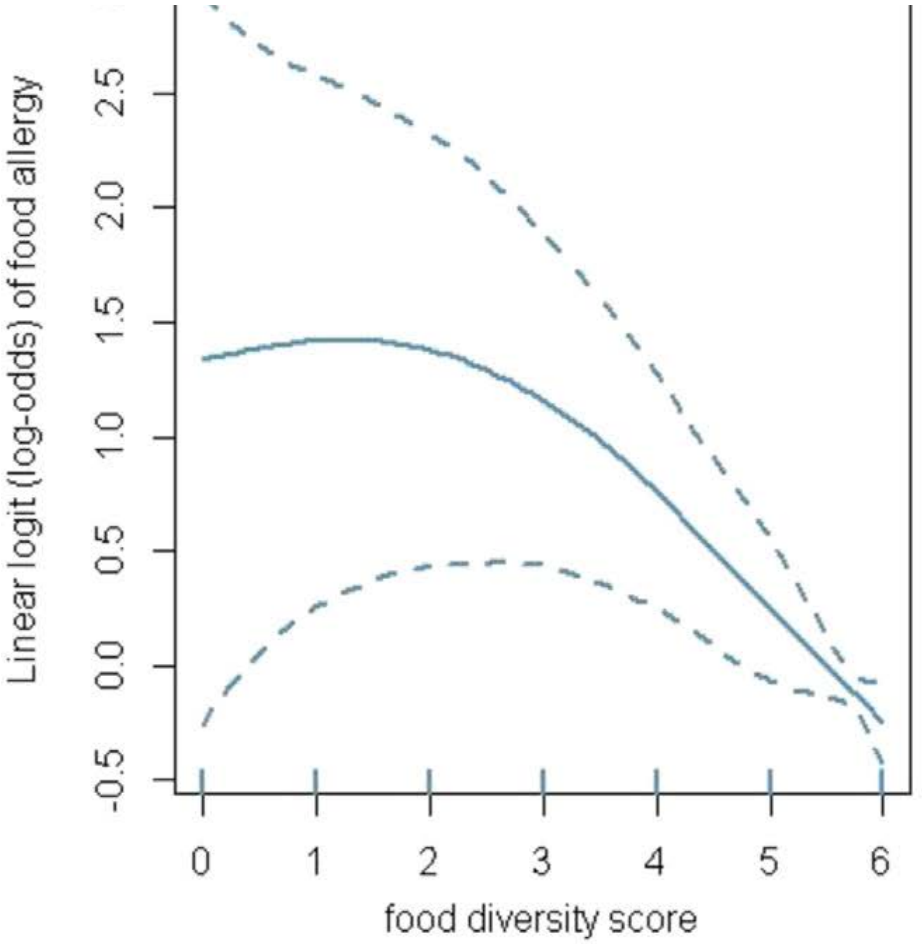



*Diversity score with major food items: vegetables or fruits, any cereals, meat, bread, cake and yogurt

Roduit et al. JACI 2012

# Early Life Food Diversity



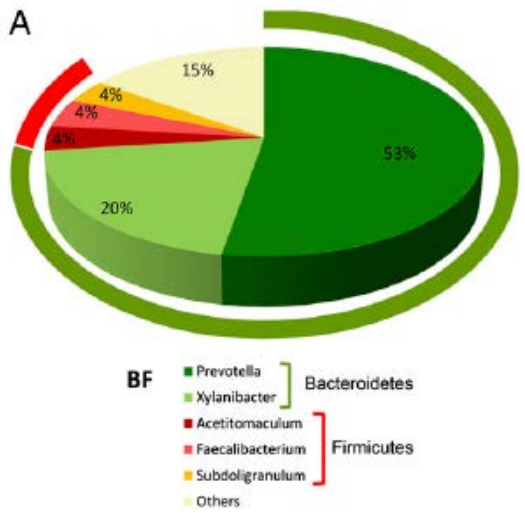

*Diversity score with major food items: vegetables or fruits, any cereals, meat, bread, cake and yogurt

Roduit et al. JACI 2014

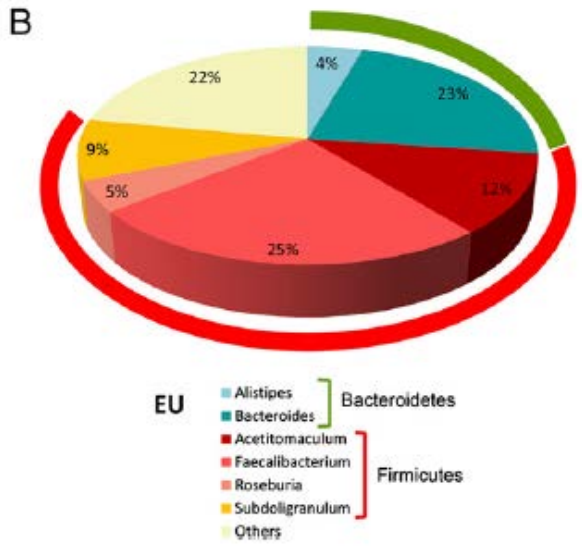
# Diet and gut microbiota

Comparison of the fecal microbiota of European children (EU) and that of children from a rural African village of Burkina Faso (BF), where the diet is high in fiber content

rural African village of Burkina Faso



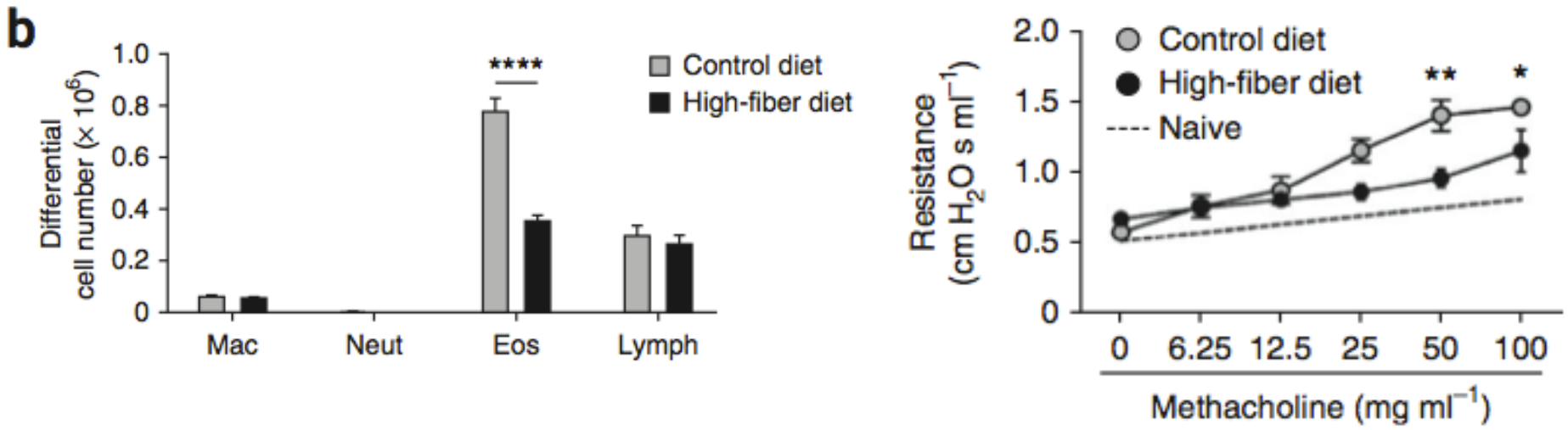

European children




De Filippo C, et al. Proc Natl Acad Sci U S A 2010

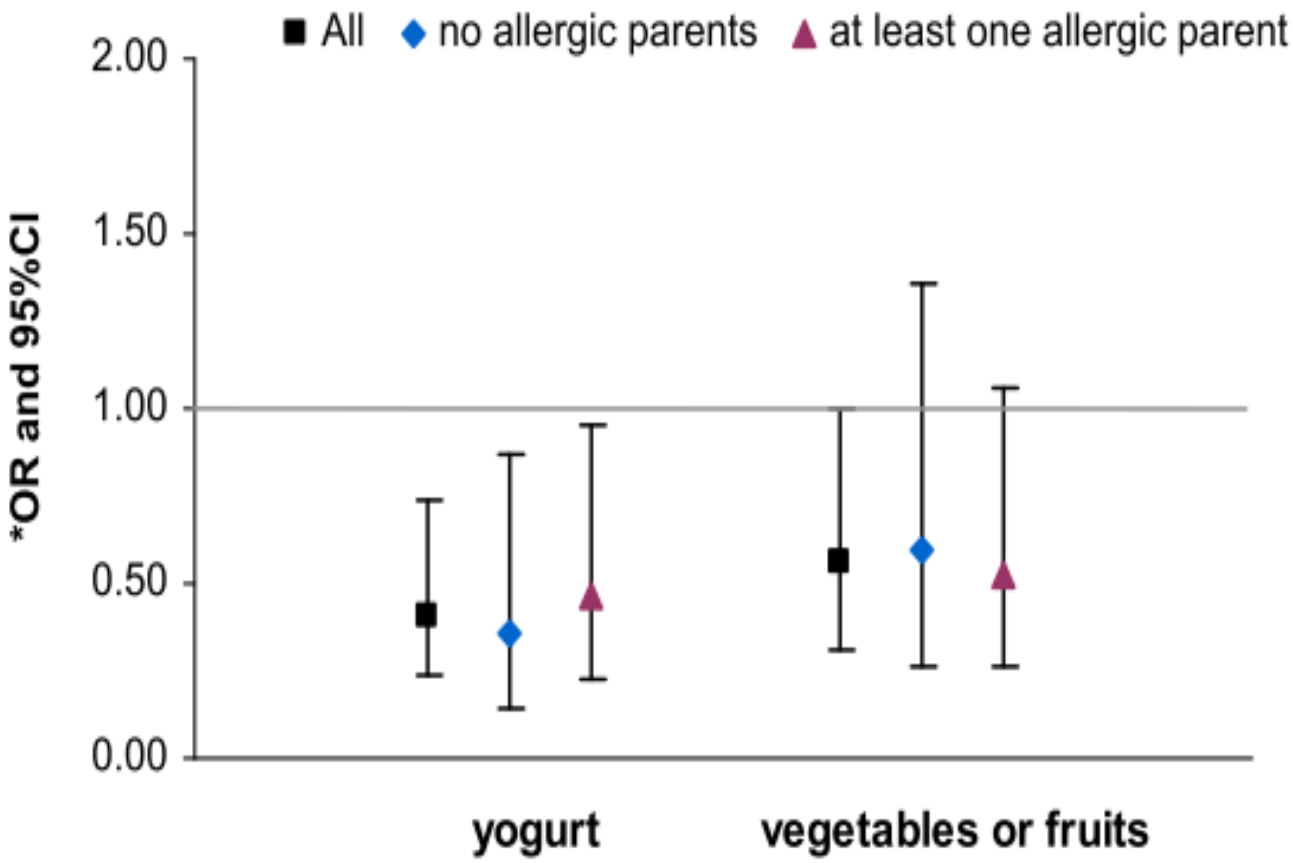
# Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis

Aurélien Trompette[1], Eva S Gollwitzer[1], Koshika Yadava[1], Anke K Sichelstiel[1], Norbert Sprenger[2], Catherine Ngom-Bru[2], Carine Blanchard[2], Tobias Junt[3], Laurent P Nicod[1], Nicola L Harris[4] & Benjamin J Marsland[1]




Trompette et al. Nature medicine 2014

# Infant diet and atopic dermatitis




*adjusted for farmer, center breastfeeding, parental history of allergies

Roduit et al. JACI 2012

# Consumption of milk products in the first year of life and asthma

**Results from the PASTURE/EFRAIM birth cohort:**

| | Asthma |
|---|---|
| | OR* and 95% CI |
| Yogurt | |
| 3-12mo | 0.47 (0.26-0.84) |
| >12mo, ref | 1 |
| Other milk products | |
| 3-12mo | 0.37 (0.22-0.64) |
| >12mo, ref | 1 |
| Butter | |
| 3-12mo | 0.45 (0.26-0.77) |
| >12mo, ref | 1 |

adjusted for centre, farmer, parents with allergy, maternal education, gender, breastfeeding, siblings, atopic dermatitis up to 6 yrs and maternal education

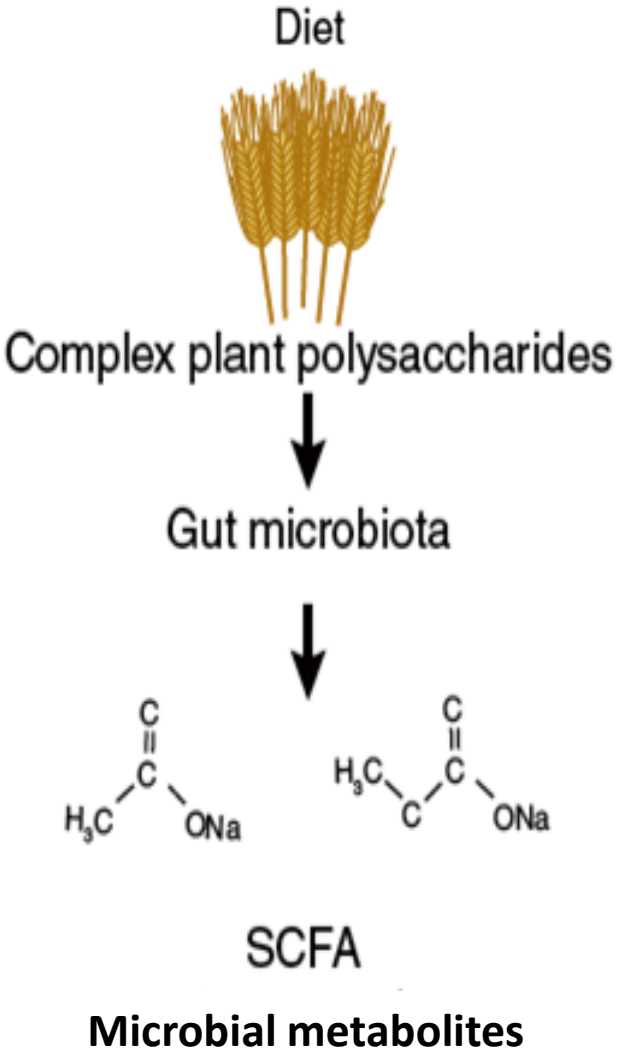
Roduit et. al, JACI 2014

# Short Chain Fatty Acids (SCFAs): Acetate, Propionate, Butyrate

2 main sources from the diet:

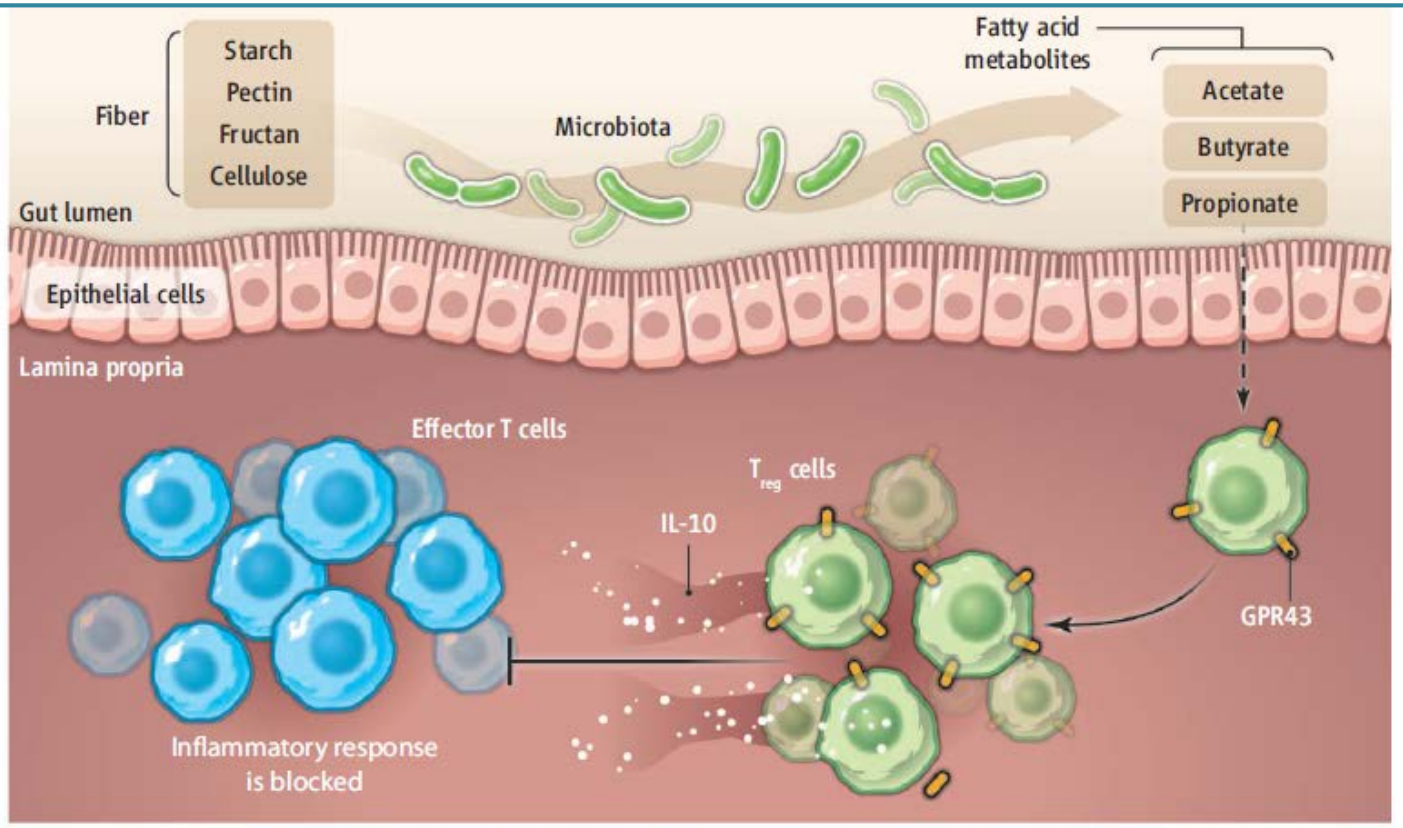
1) Eating fiber: the gut microbiota will metabolize it by fermentation into SCFAs

2) Present in butter or yogurt (Butyrate)



IMMUNOLOGY

# Feed Your $T_{regs}$ More Fiber

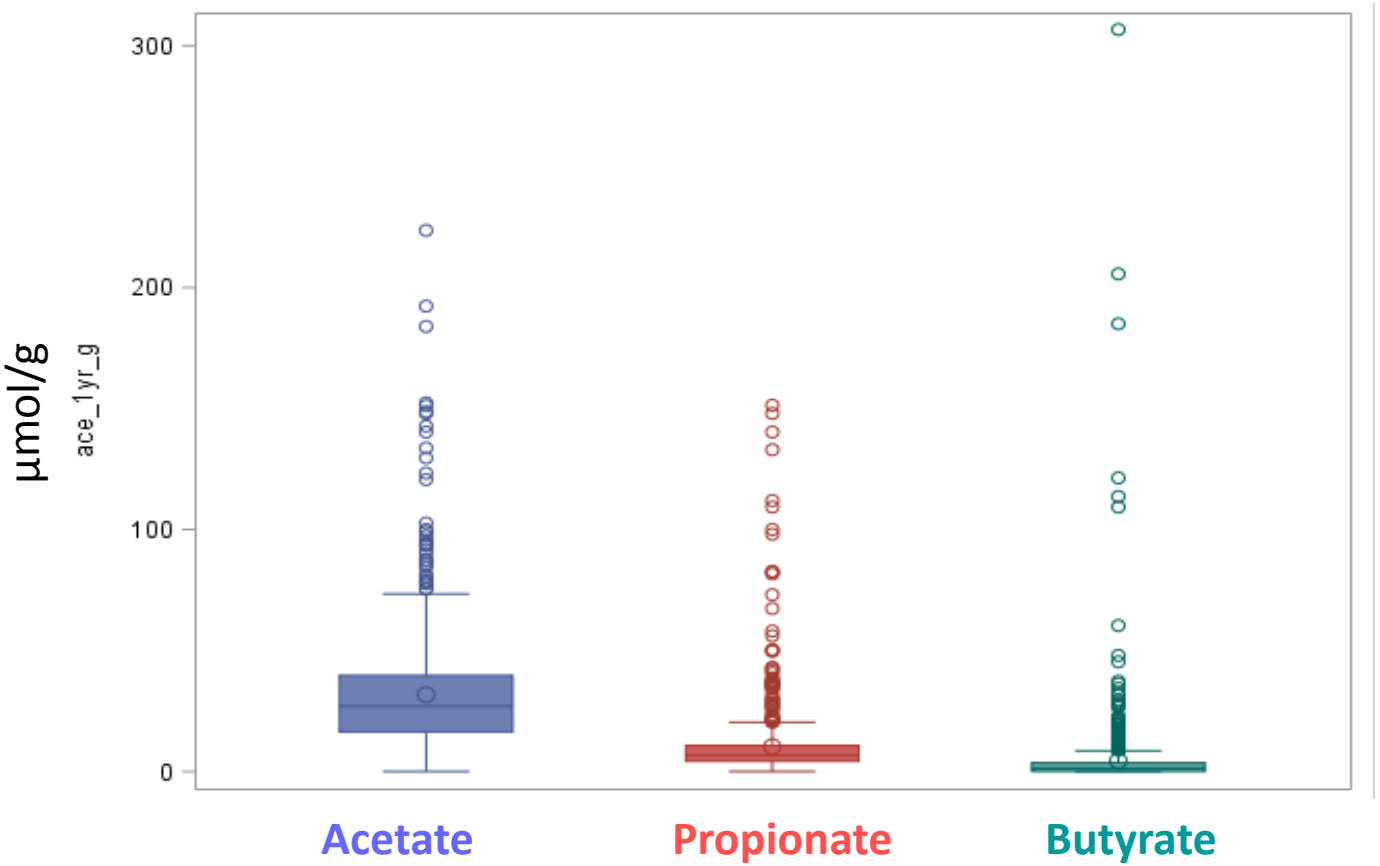



**Bacterial metabolites fight intestinal inflammation.** Commensal bacteria metabolize fiber and generate short-chain fatty acids. These fatty acids are ligands for GPR43 expressed by $T_{reg}$ cells and stimulate their expansion and immune-suppressive properties such as the production of IL-10, thereby controlling proinflammatory responses in the gut.

- **Major metabolites produced by bacteria in the gut are short chain fatty acids (SCFAs). These metabolites have strong anti-inflammatory effects, inducing regulatory T cells.**

Julia Bollrath and Fiona Powrie, Science 2013

# SCFA levels in fecal samples of children: PASTURE study




| SCFAs (umol/g) | N | Mean | Std Dev | Minimum | Maximum | N; % of 0 |
|---|---|---|---|---|---|---|
| Acetate | 778 | 31.7 | 24.5 | 0 | 223.7 | 3; 0.4% |
| Propionate | 778 | 10.2 | 15.1 | 0 | 151.3 | 32; 4% |
| Butyrate | 778 | 4.4 | 17.1 | 0 | 306.7 | 188; 24% |

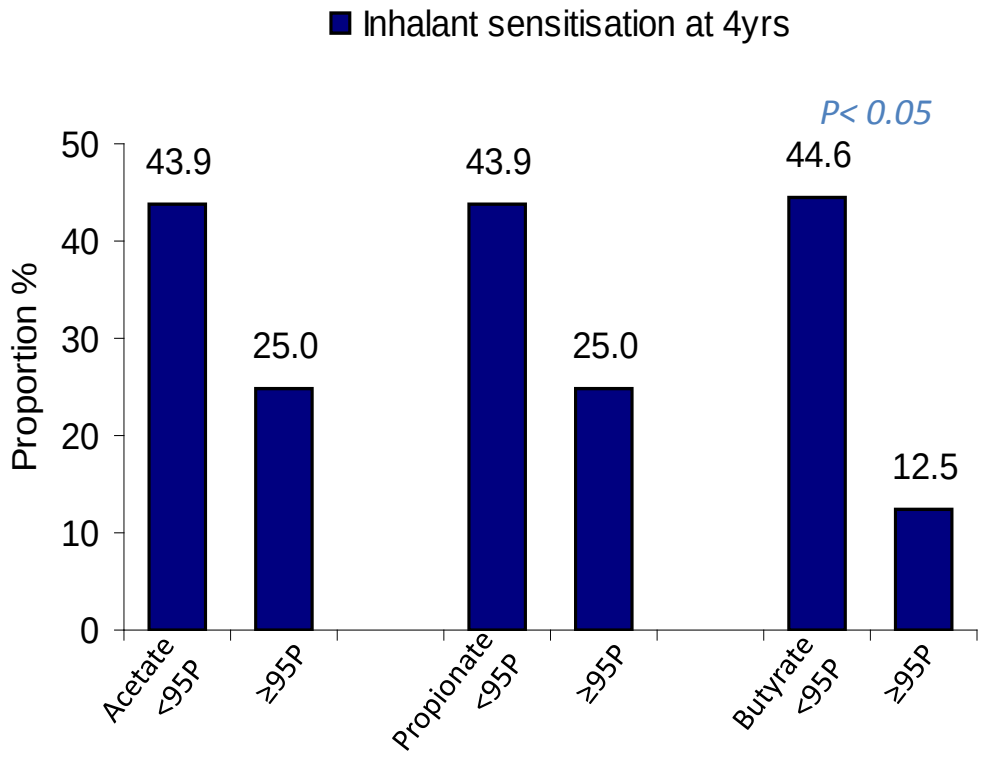
# Health outcomes depending on the level of butyrate

| *Health outcomes* | Butyrate < 97P (<20.9 µmol/g) n=754 | | Butyrate ≥ 97P (≥20.9 µmol/g) n=24 | |
|---|---|---|---|---|
| | **n** | **%** | **n** | **%** |
| **Asthma up to 6 years** | 57 | 9.1 | 1 | 5.0 |
| | | | | |
| **Allergic rhinitis up to 6 years** | 52 | 7.9 | 1 | 4.8 |
| | | | | |
| **Atopic dermatitis up to 6 years** | 201 | 32.3 | 9 | 45.0 |
| | | | | |
| **Food allergy up to 3 years** | 43 | 6.5 | 1 | 4.6 |
| | | | | |
| **Food sensitization at 1y (cut off 0.7)** | 26 | 3.8 | 0 | 0.0 |
| | | | | |
| **Inhalant sensitization at 1y (cut off 0.7)** | 106 | 15.7 | 3 | 14.3 |

# Level of SCFA and exposures

| *Exposures* | Butyrate < 97P (<20.9 µmol/g) n=754 | | Butyrate ≥ 97P (≥20.9 µmol/g) n=24 | |
|---|---|---|---|---|
| | n | % | n | % |
| Farmer | 351 | 46.6 | 12 | 50.0 |
| | | | | |
| Contact to pets (cats or dogs) in 1st year | 425 | 56.5 | 12 | 50.0 |
| | | | | |
| Antibiotic during pregnancy | 174 | 23.2 | 2 | 8.3 |
| | | | | |
| Farm animal contact during pregnancy: | | | | |
| 3 or 4 species | 83 | 11.5 | 3 | 13.6 |
| 1 or 2 species | 343 | 47.4 | 12 | 54.6 |
| 0 | 298 | 41.2 | 7 | 31.8 |
| | | | | |
| Nutrition 1st year: | | | | |
| Cow's milk: yes vs no | 403 | 53.4 | 15 | 62.5 |
| Yogurt: yes vs no | 604 | 80.8 | 17 | 70.8 |
| Other milk products: yes vs no | 551 | 73.7 | 19 | 79.2 |
| Butter: yes vs no | 522 | 69.8 | 20 | 83.3 |
| Margarine: yes vs no | 455 | 60.8 | 11 | 45.8 |
| Egg: yes vs no | 497 | 66.4 | 16 | 66.7 |
| Fish: yes vs no | 444 | 59.4 | 14 | 58.3 |
| Meat (in first 9 months): yes vs no | 514 | 68.7 | 13 | 54.2 |
| Cereals (in first 9 months):yes vs no | 503 | 67.3 | 15 | 62.5 |
| Vegetables/fruits (in first 6 mo): yes vs no | 343 | 45.9 | 7 | 29.2 |

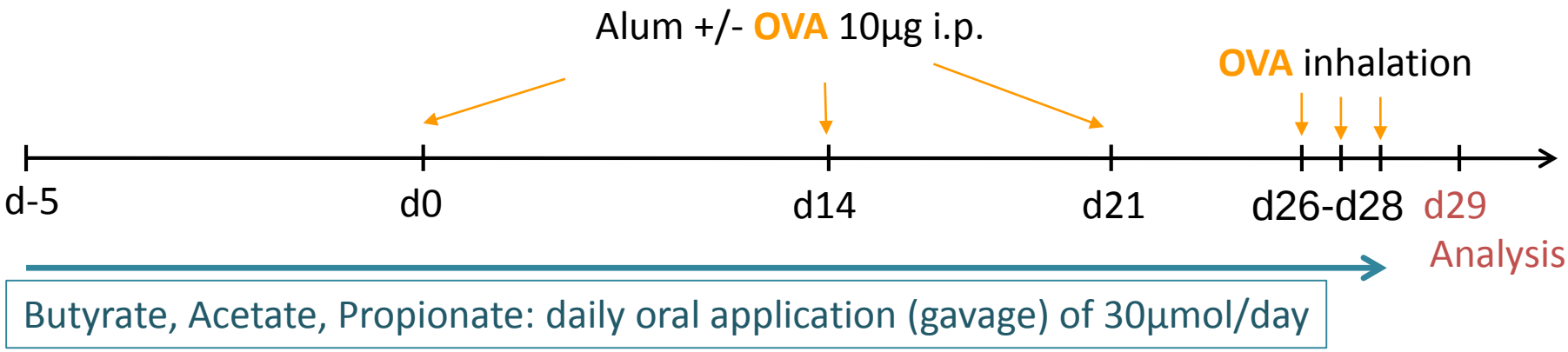
# SCFAs and Allergen Sensitization




**(95P= 95th percentile)**

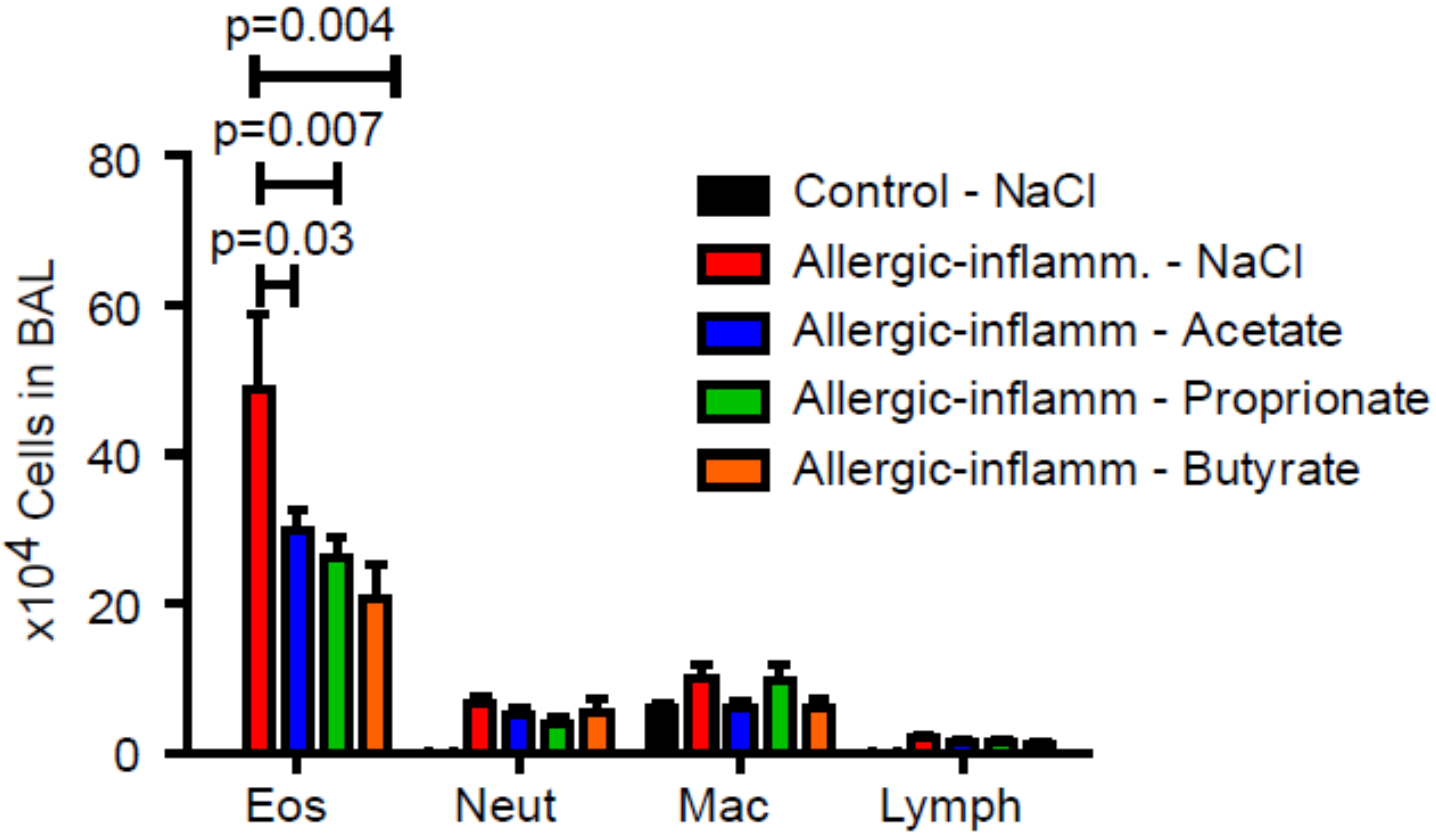
*Roduit & Frei et al., in preperation*

# Allergic airway inflammation model (ovalbumin, OVA) with SCFA application

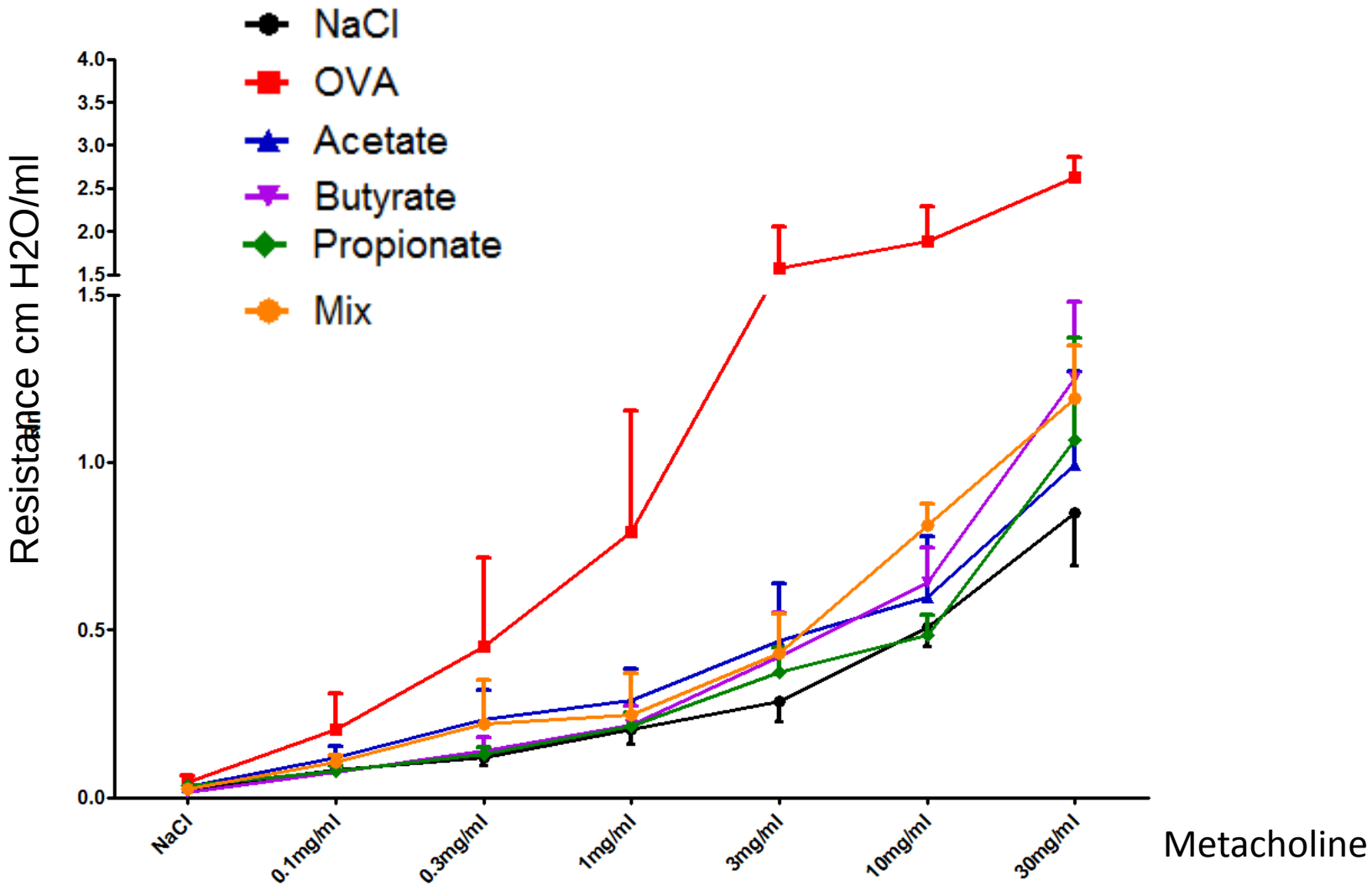



Butyrate, Acetate, Propionate: daily oral application (gavage) of 30µmol/day

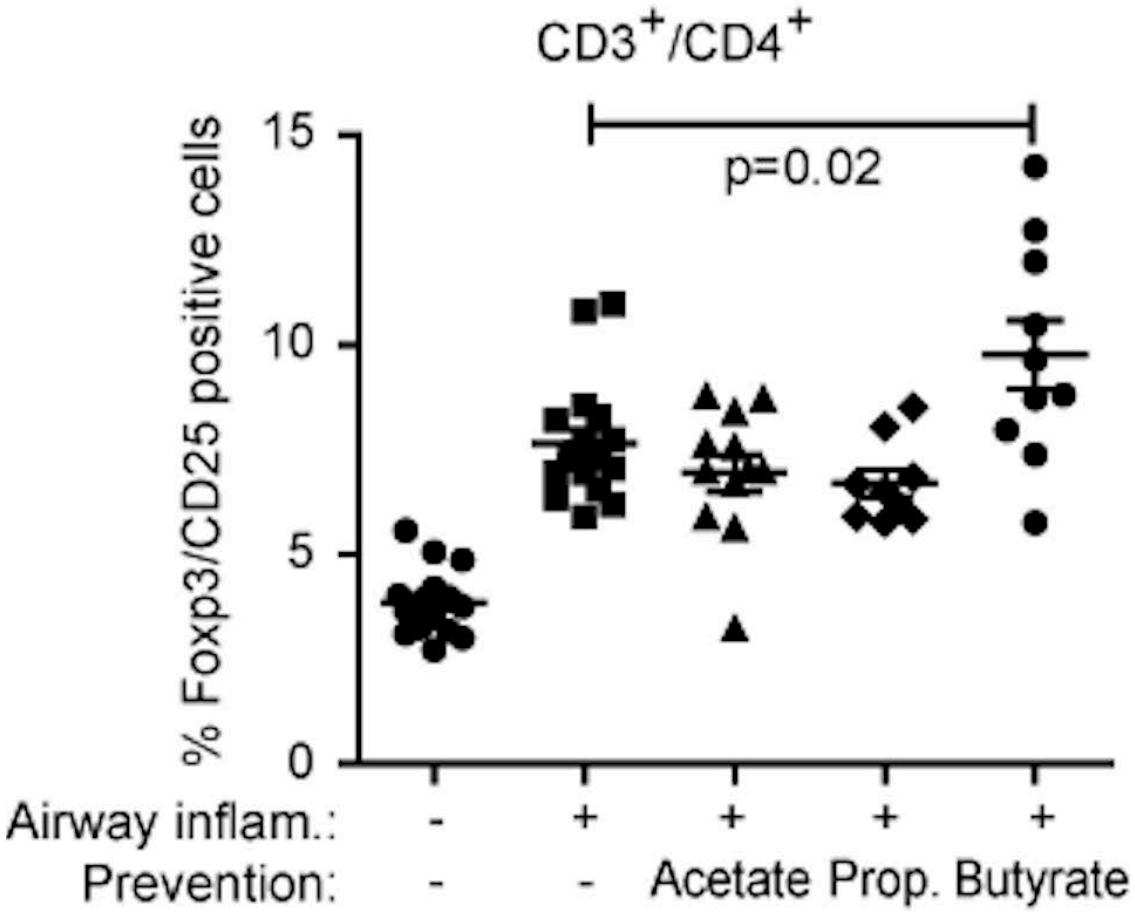
# Application of SCFA reduces BAL cells counts



# Application of SCFA reduces airway hyper-reactivity

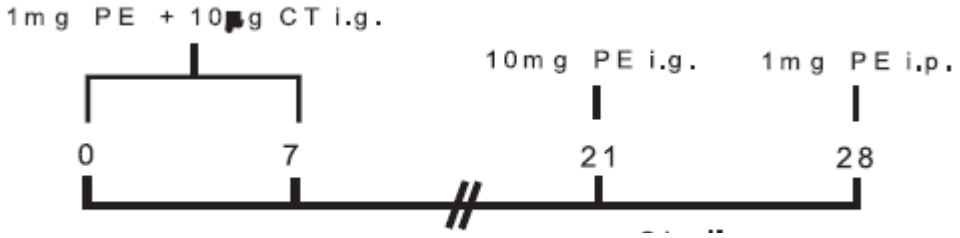


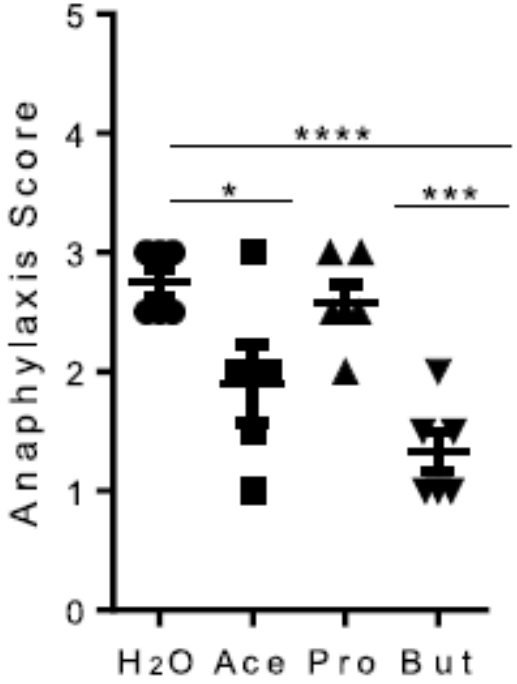
# T regulatory cells in the lungs



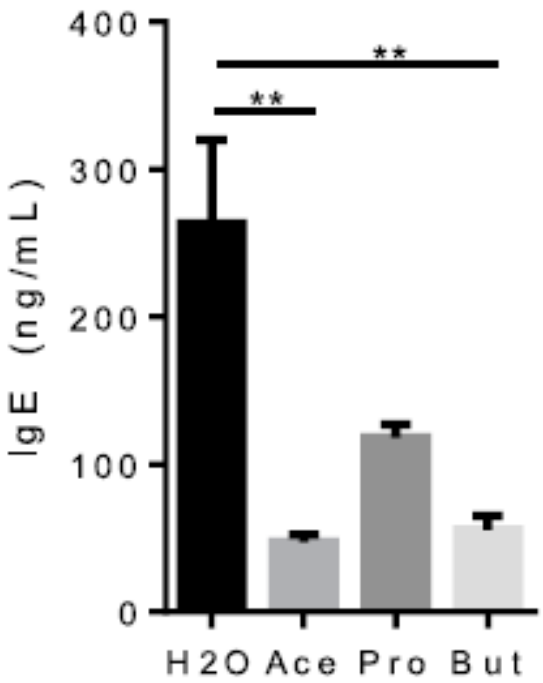

*Roduit & Frei et al., in preperation*

# SCFA enhances oral tolerance and protects against food allergy

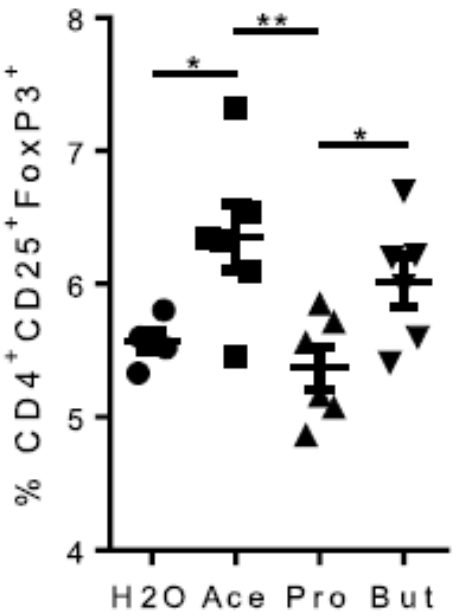



Anaphylaxis score at day 28




Total IgE in serum




Treg cells

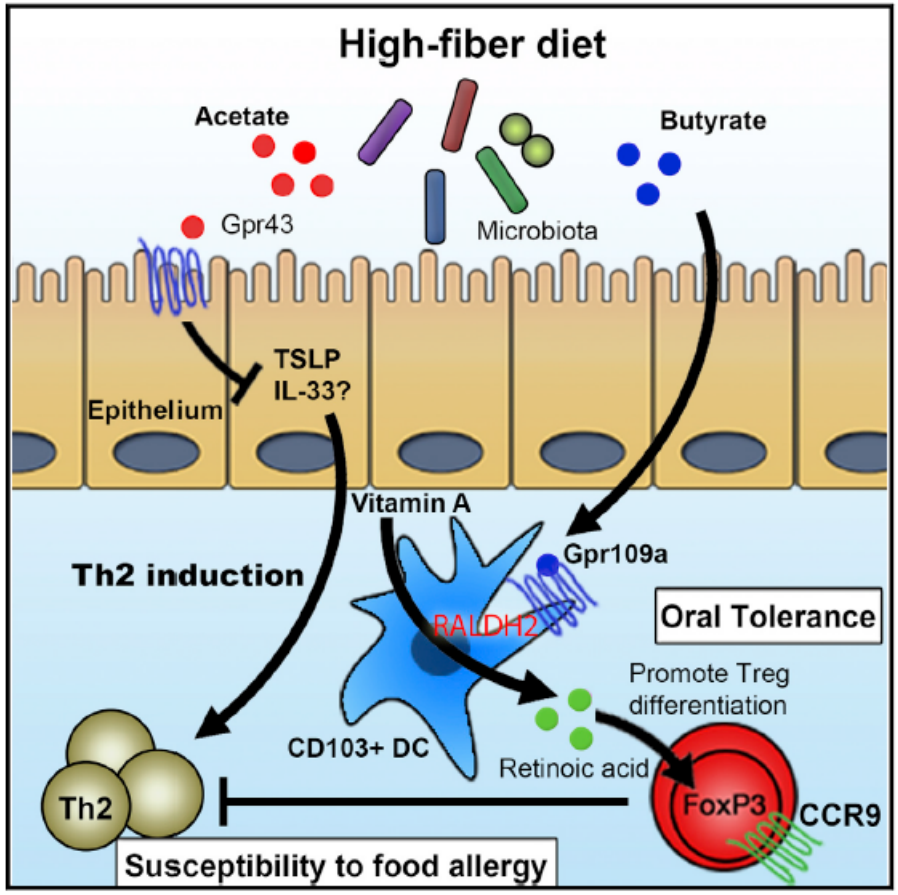
Tan, J. et al. Cell Reports 2016

Cell Reports



# Dietary Fiber and Bacterial SCFA Enhance Oral Tolerance and Protect against Food Allergy through Diverse Cellular Pathways

## Graphical Abstract

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## Correspondence

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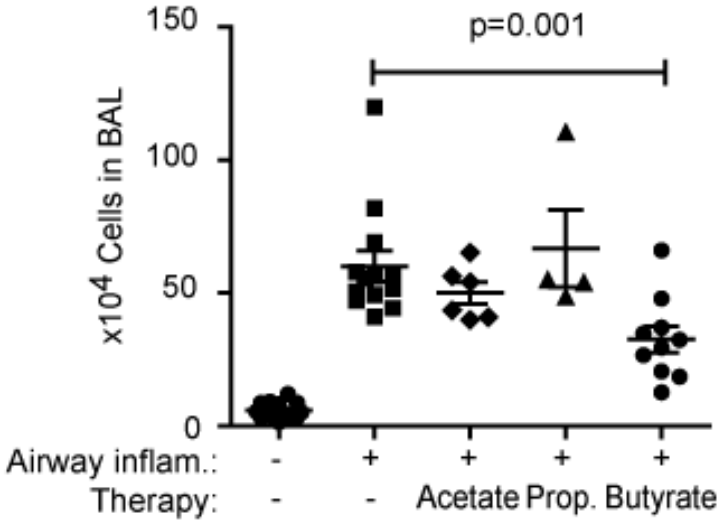
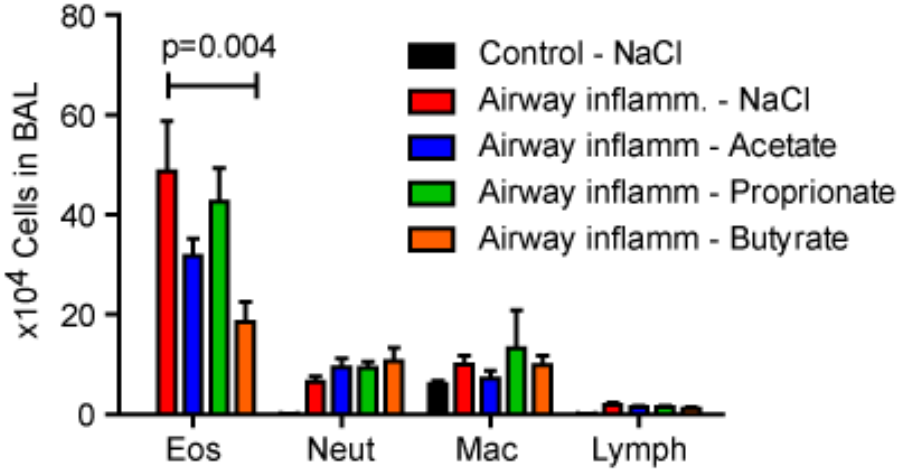
## In Brief

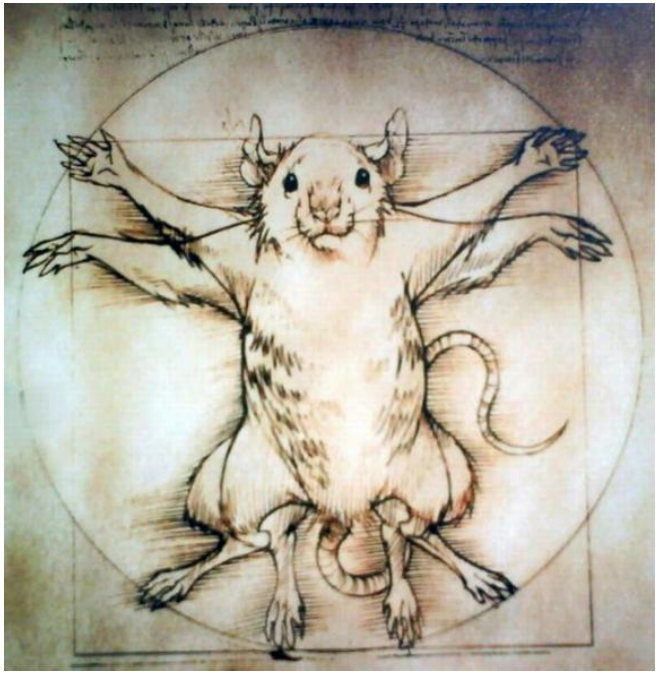
Tan et al. examine the beneficial roles of dietary fiber in peanut allergy using mice. The authors find that this effect involves reshaping of the gut microbiota as well as increased levels of short-chain fatty acids and activity of their receptors GPR43 and GPR109a. High-fiber feeding also increased tolerogenic CD103$^{+}$ DCs activity, leading to increased Treg cell differentiation.

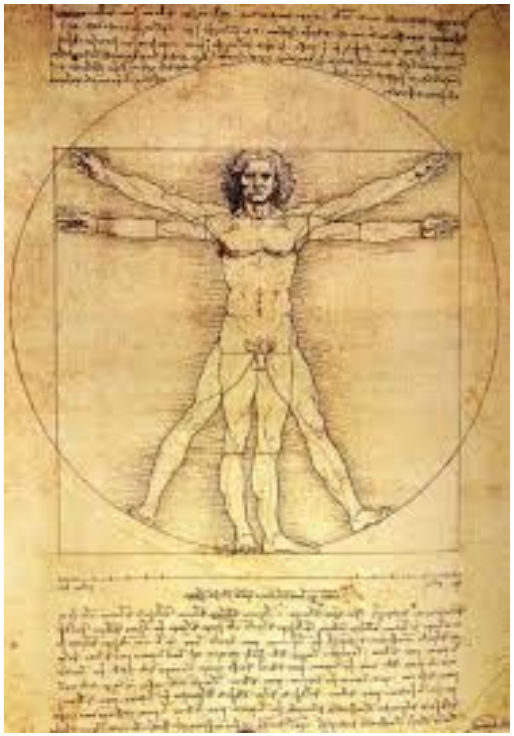
# Therapy versus Prevention



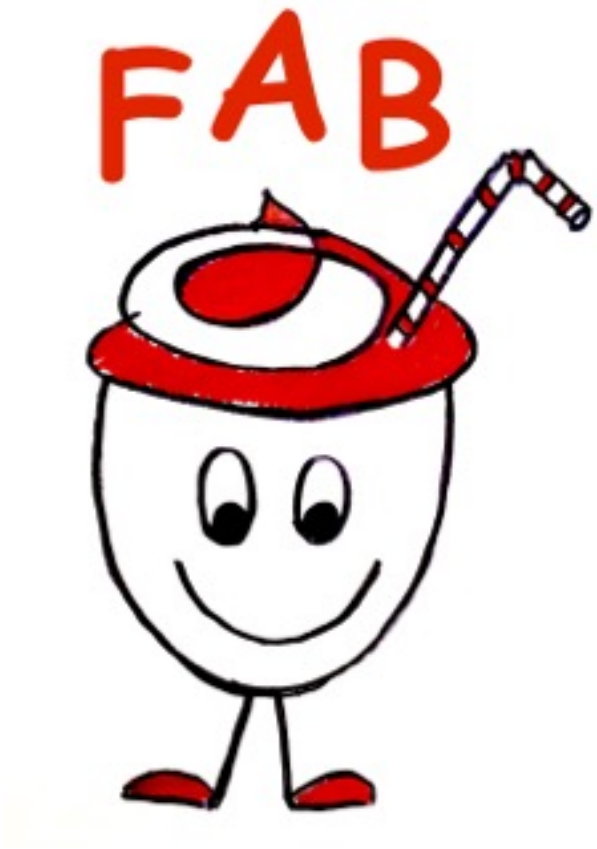




*Roduit & Frei et al., in preperation*



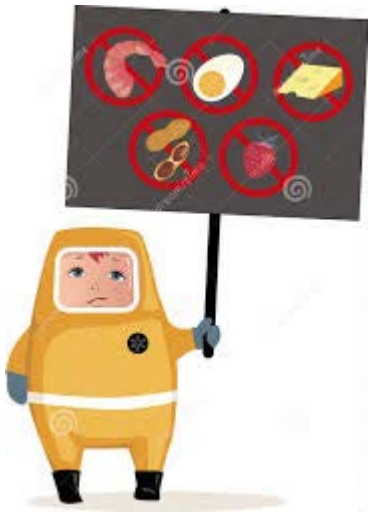


# FAB-Study
# **F**ood **A**llergy treatment with **B**utyrate



# FAB – Study: interventional study on food allergy

- *Hypothesis:* Butyrate will help to improve oral tolerance to incriminated food protein
- *Objective:* Diet rich in Butyrate to increase oral tolerance in children
- *Method*: daily milk-shake (1,5g-2g Butyrate)

# Study design

- Children, 4-12 years old, with food allergy (no milk product allergy), at least for 3 weeks at HGK
- In addition to daily food: daily milk-shake
- Control-group: Shake made from non cow's milk
- Single blinded study
- 31 children per group

# Analysis of FAB Study

- LOAL (lowest observable adverse event level ) beginning - end
- Fecal samples: beginning – middle – end (SCFA content)
- Daily documentation: diet and symptoms
- Blood: beginning – end (IgG4, IgE, Basophil activation, Treg)

**AIM: to give advice to change diet to improve food allergy symptoms**

# Acknowledgments:


- Roger Lauener
- Caroline Roduit
- Remo Frei
- Susanne Loeliger
- Liam O'Mahony


SIAF

CK CARE

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