The gut microbiome contributes to the protective farm effect on childhood asthma

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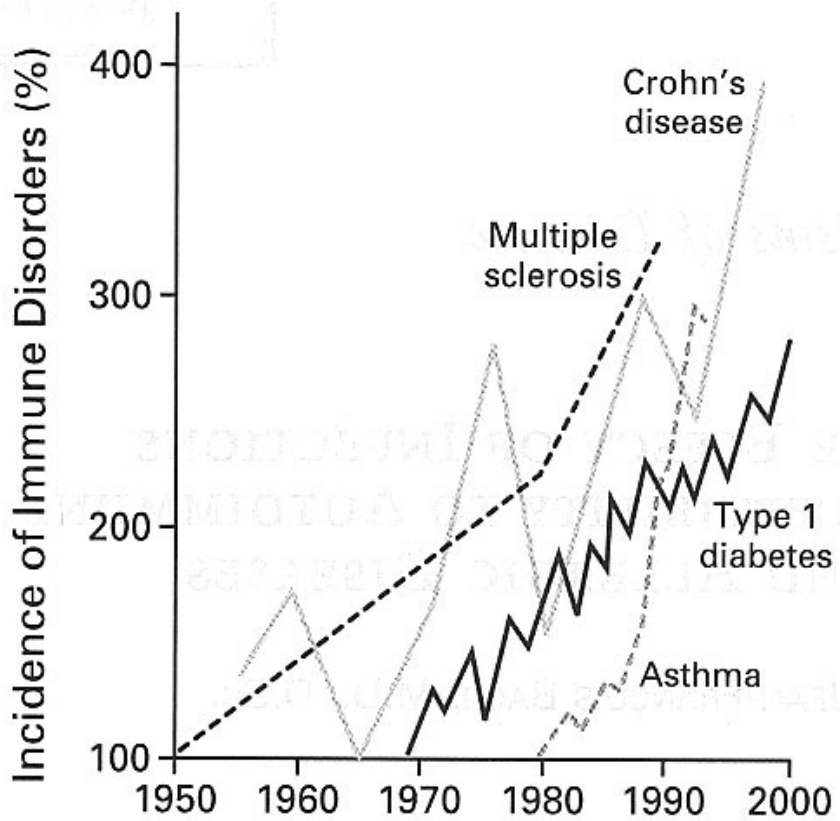
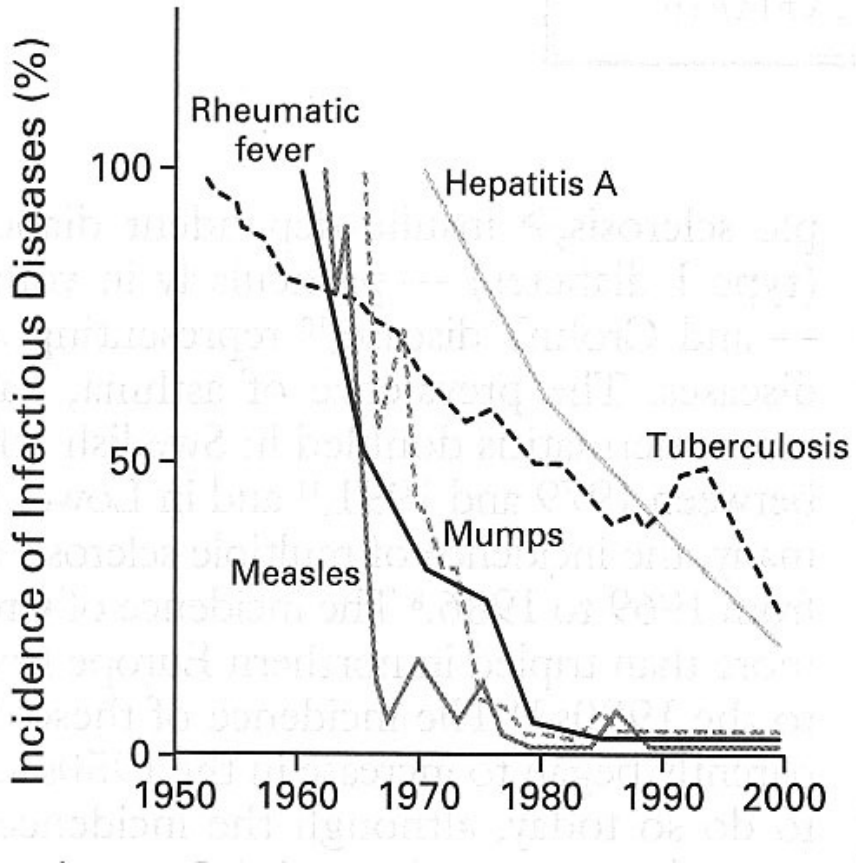
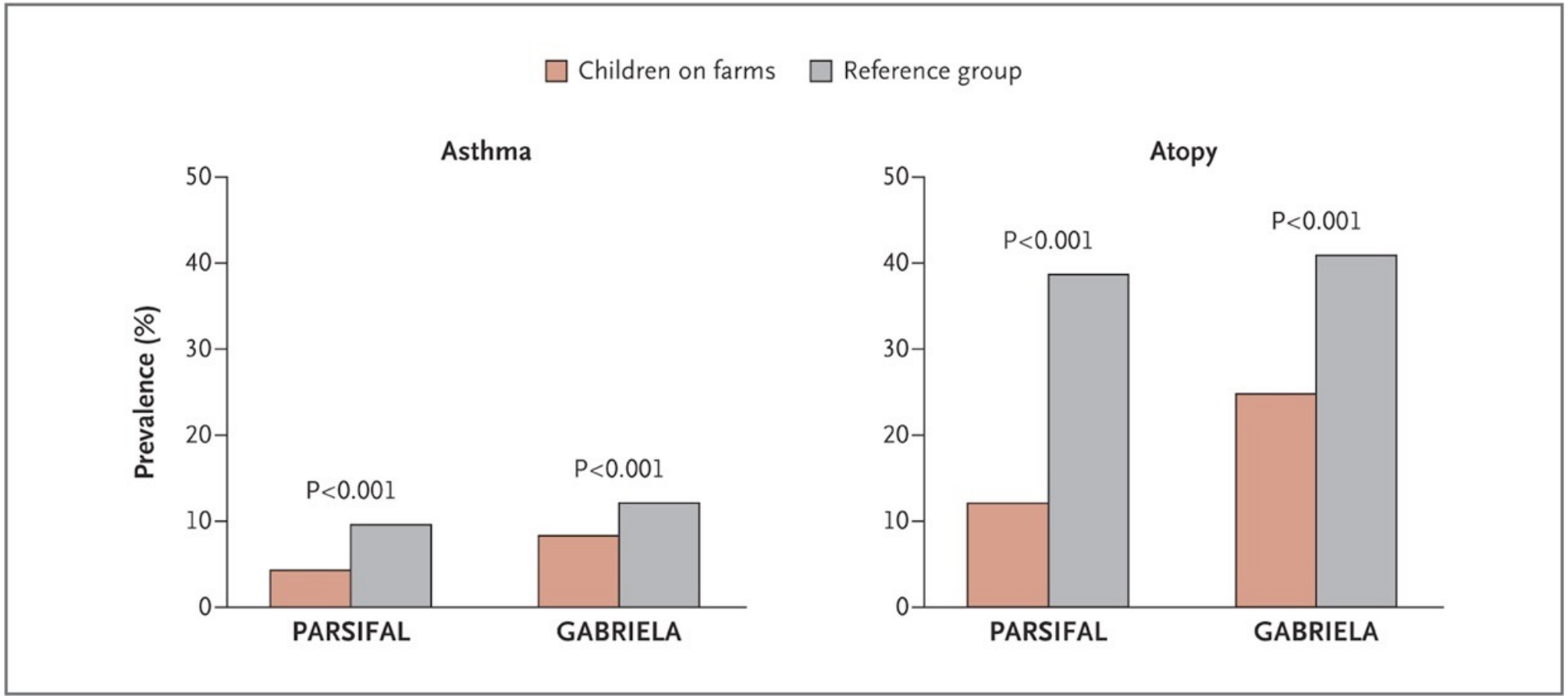
Hygiene Hypothesis and infections





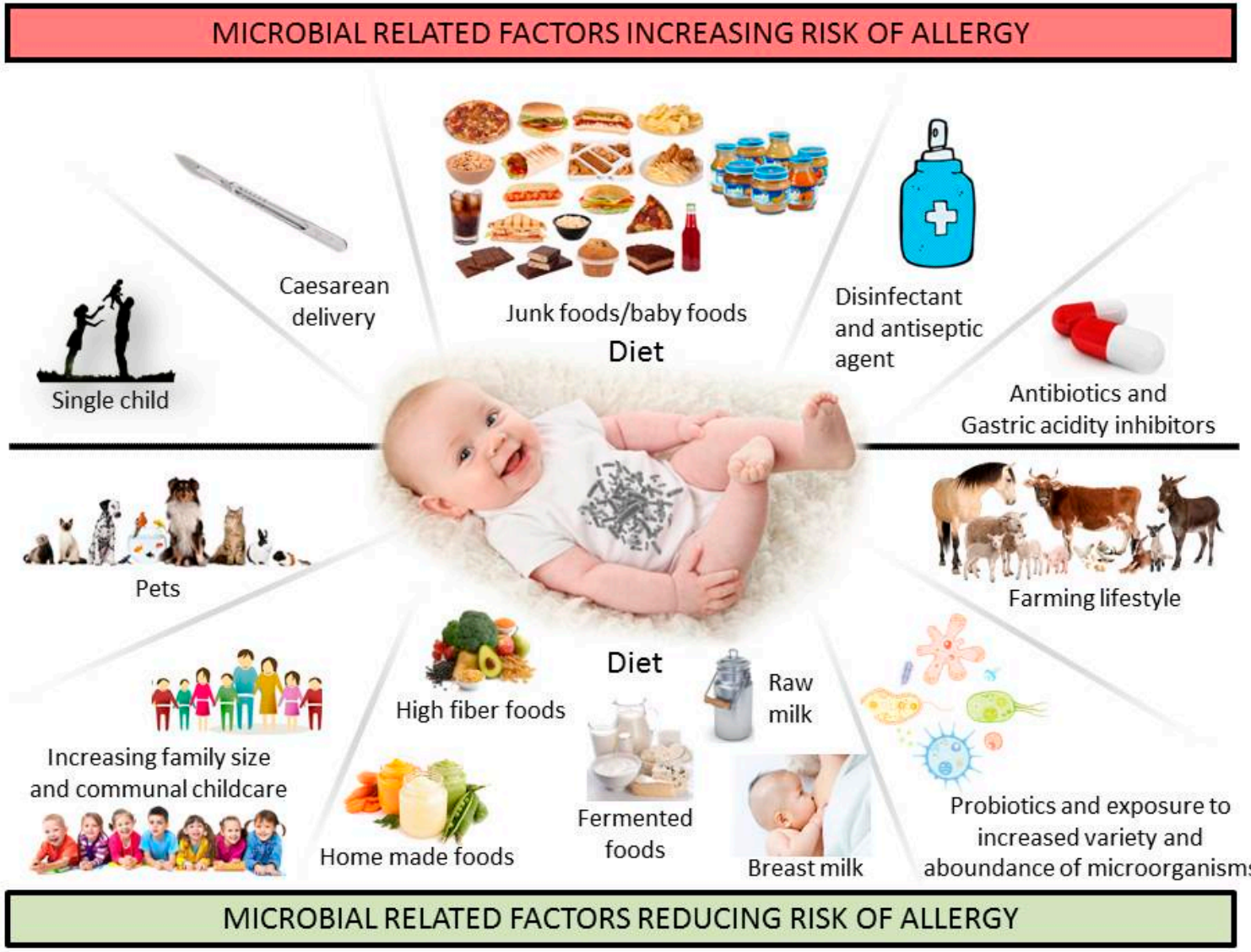
Bach J-F *N Engl J Med* 2002;347:911-20

Prevalence of Asthma and Atopy among Children Living on Farms as Compared with Reference Groups



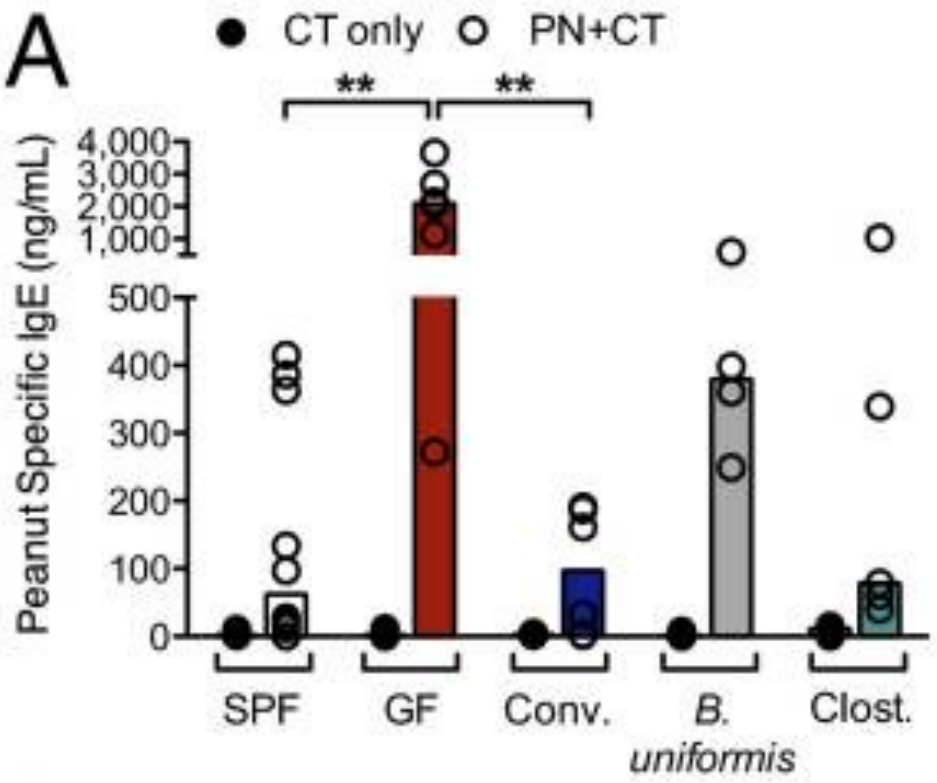
Ege et al. NEJM 2011

Hygiene Hypothesis and the microbiome



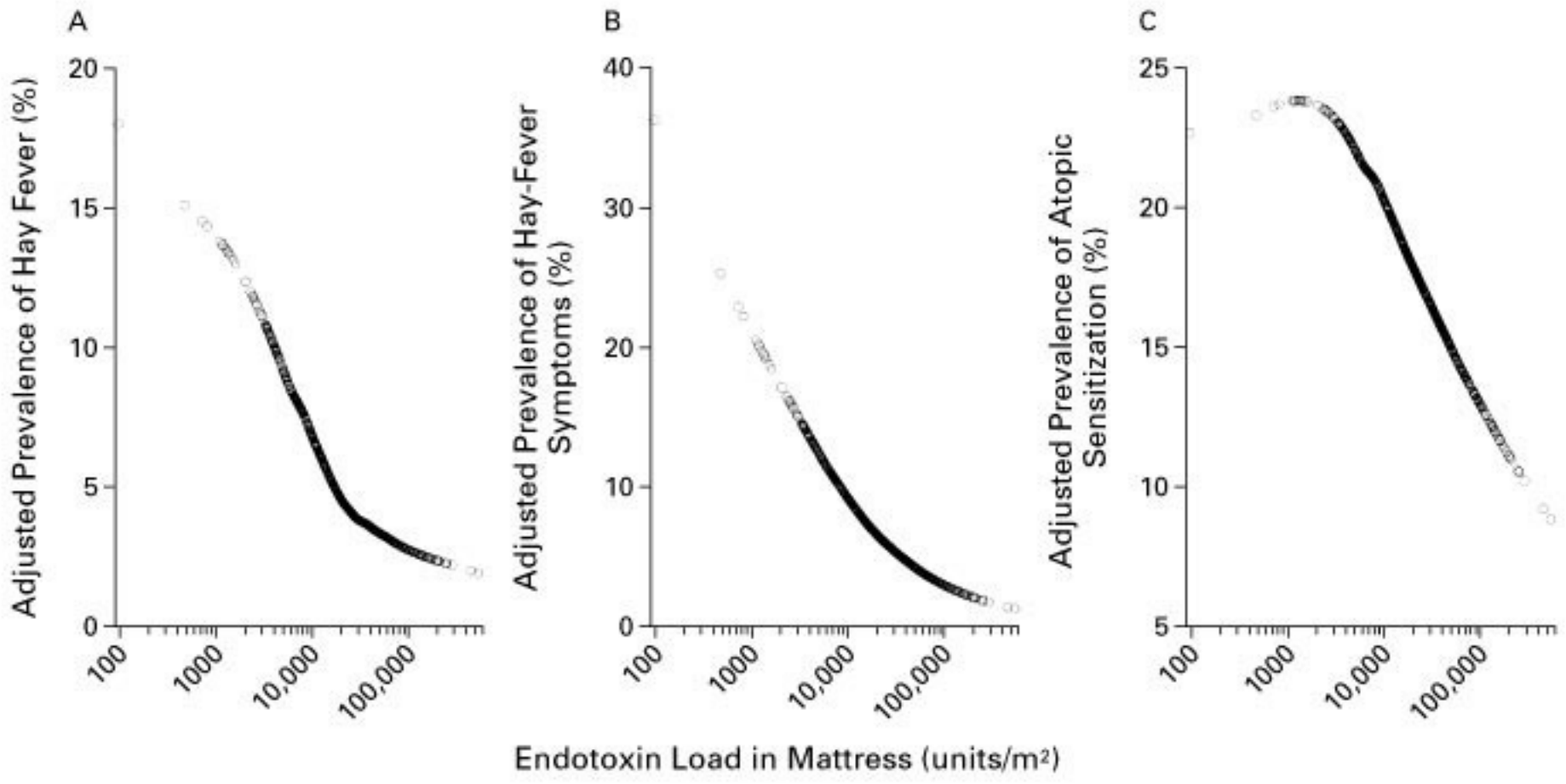
Aitoro et al. Nutrients 2017

Germ-free mice are more susceptible to food allergies than mice with commensal bacteria



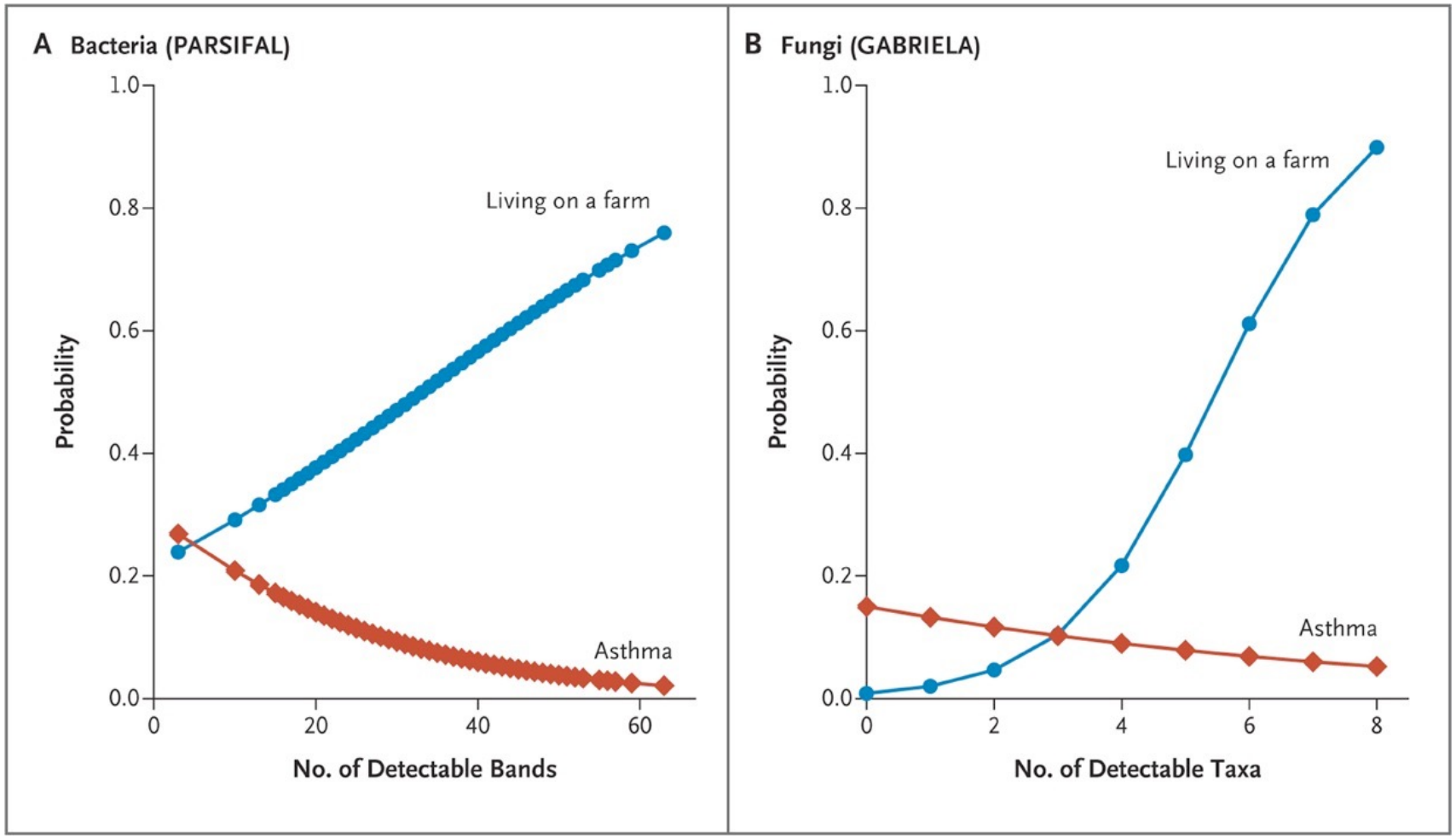
Stefka et al. PNAS 2014

Smoothed Plots of the Prevalence of Hay Fever (Panel A), Hay-Fever Symptoms (Panel B), and Atopic Sensitization (Panel C) in Relation to the Log-Transformed Endotoxin-Load Values



Braun-Farhländer et al. NEJM 2002

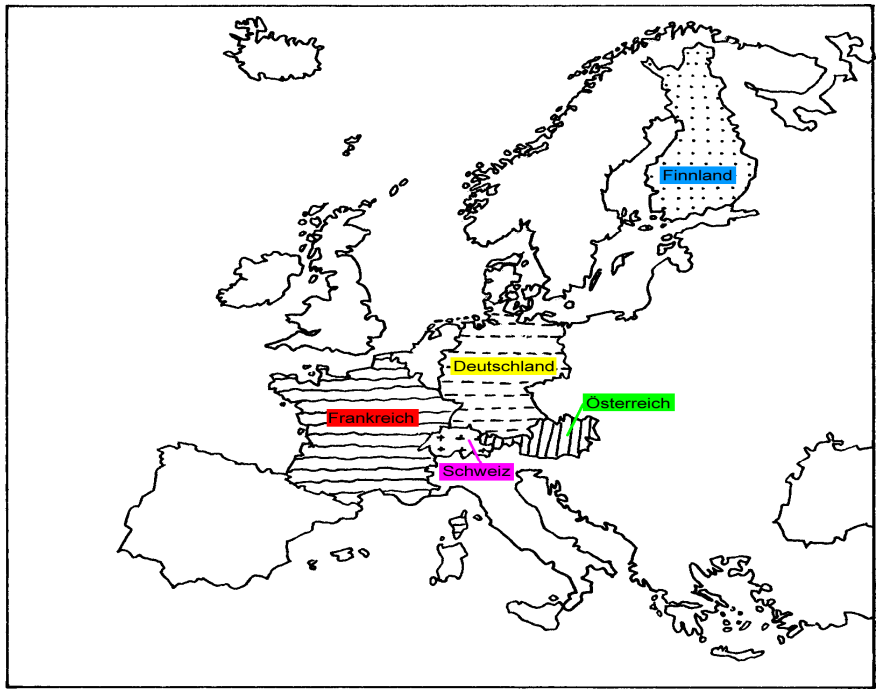
Relationship between Microbial Exposure and the Probability of Asthma



Ege et al. NEJM 2011

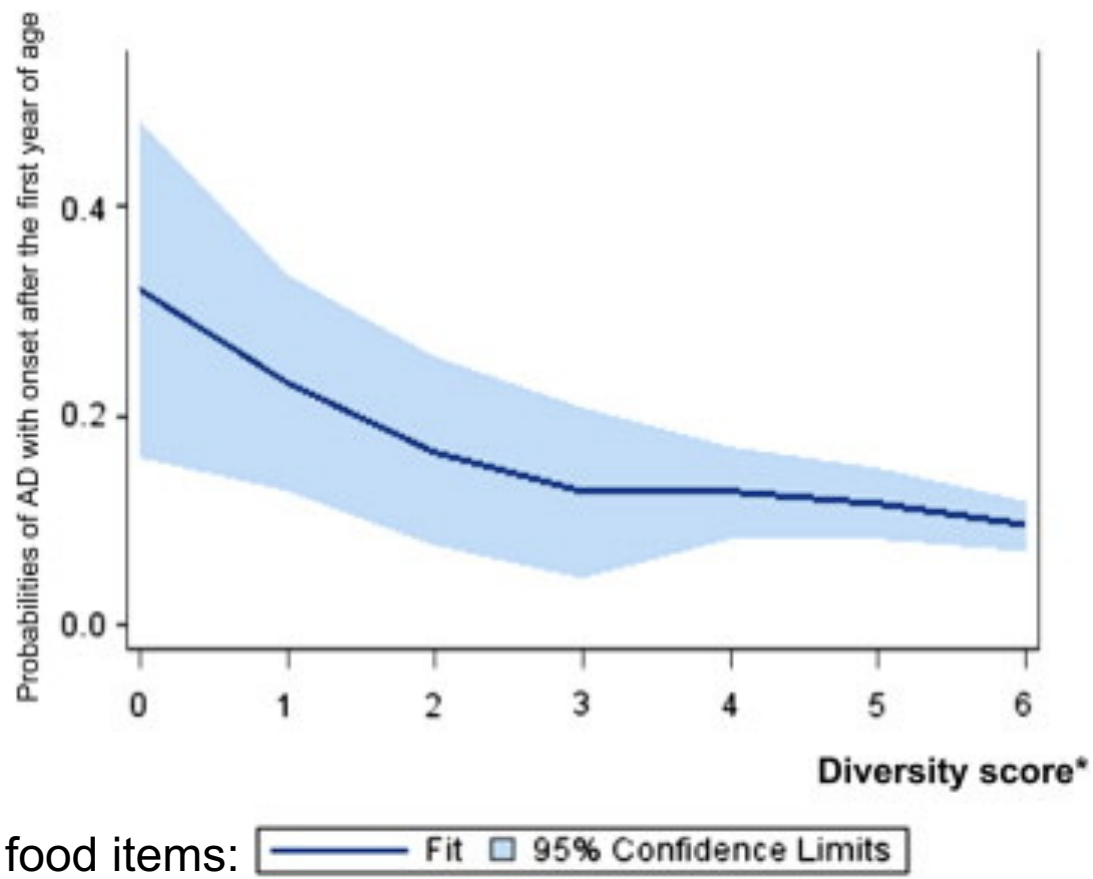
PASTURE/EFRAIM Study

- Longitudinal birth cohort study
- 500 farmer and 500 non-famer families
- 5 different European countries
- The children reached 16 years



Consumption of increasing numbers food items in the first year of life and atopic dermatitis later in life

PASTURE/EFRAIM birth cohort:

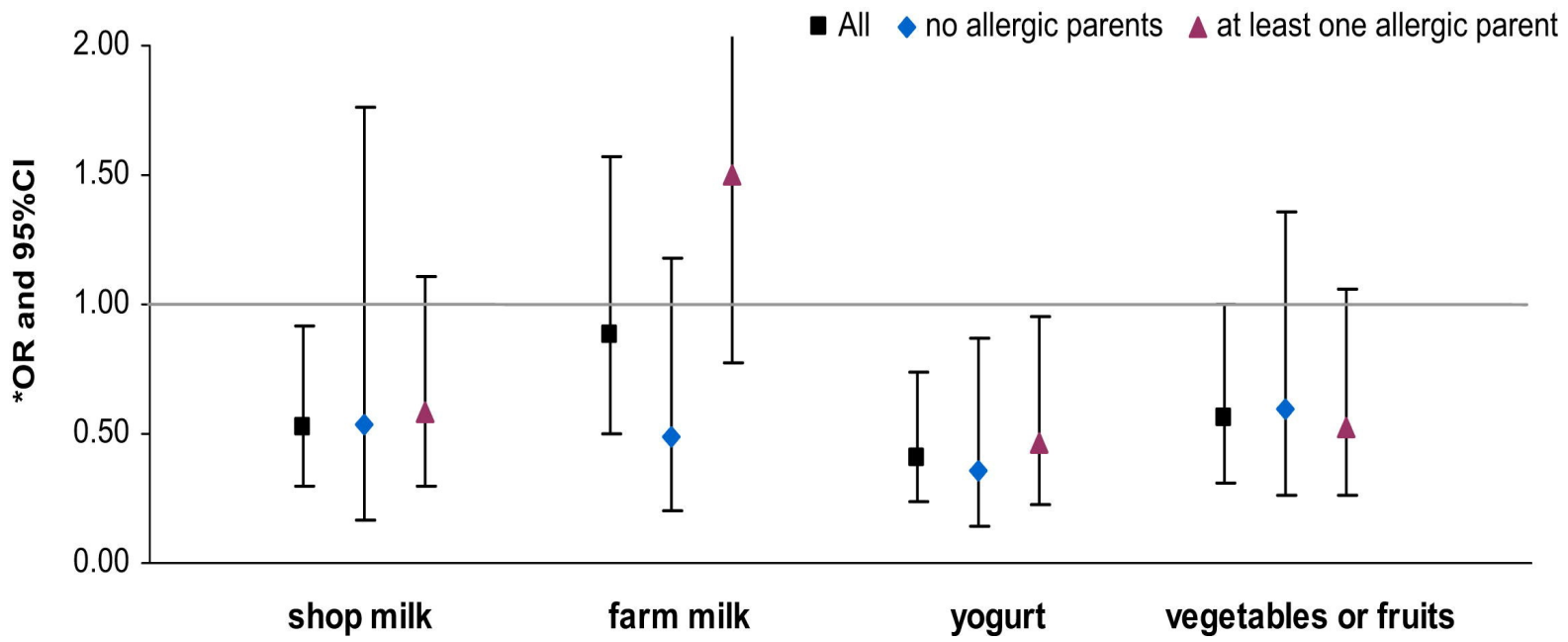


*Diversity score calculated with major food items: vegetables or fruits, any cereals, meat, bread, cake, and yogurt

Roduit et. al, JACI 2012

Consumption of food items in the first year of life and atopic dermatitis later in life

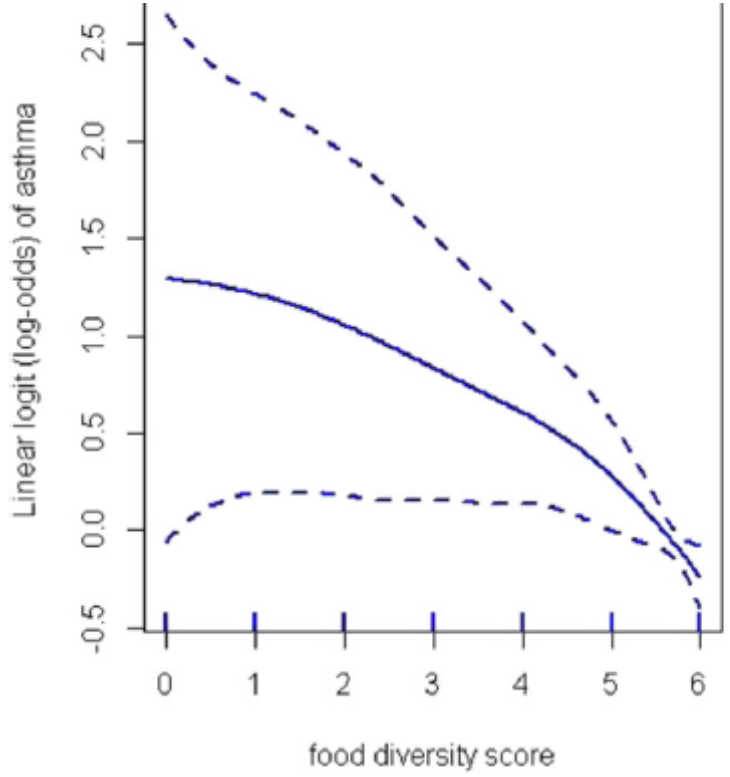
PASTURE/EFRAIM birth cohort:



Roduit et. al, JACI 2012

Consumption of increasing numbers food items in the first year of life and asthma later in life

Complementary food introduction in newborn

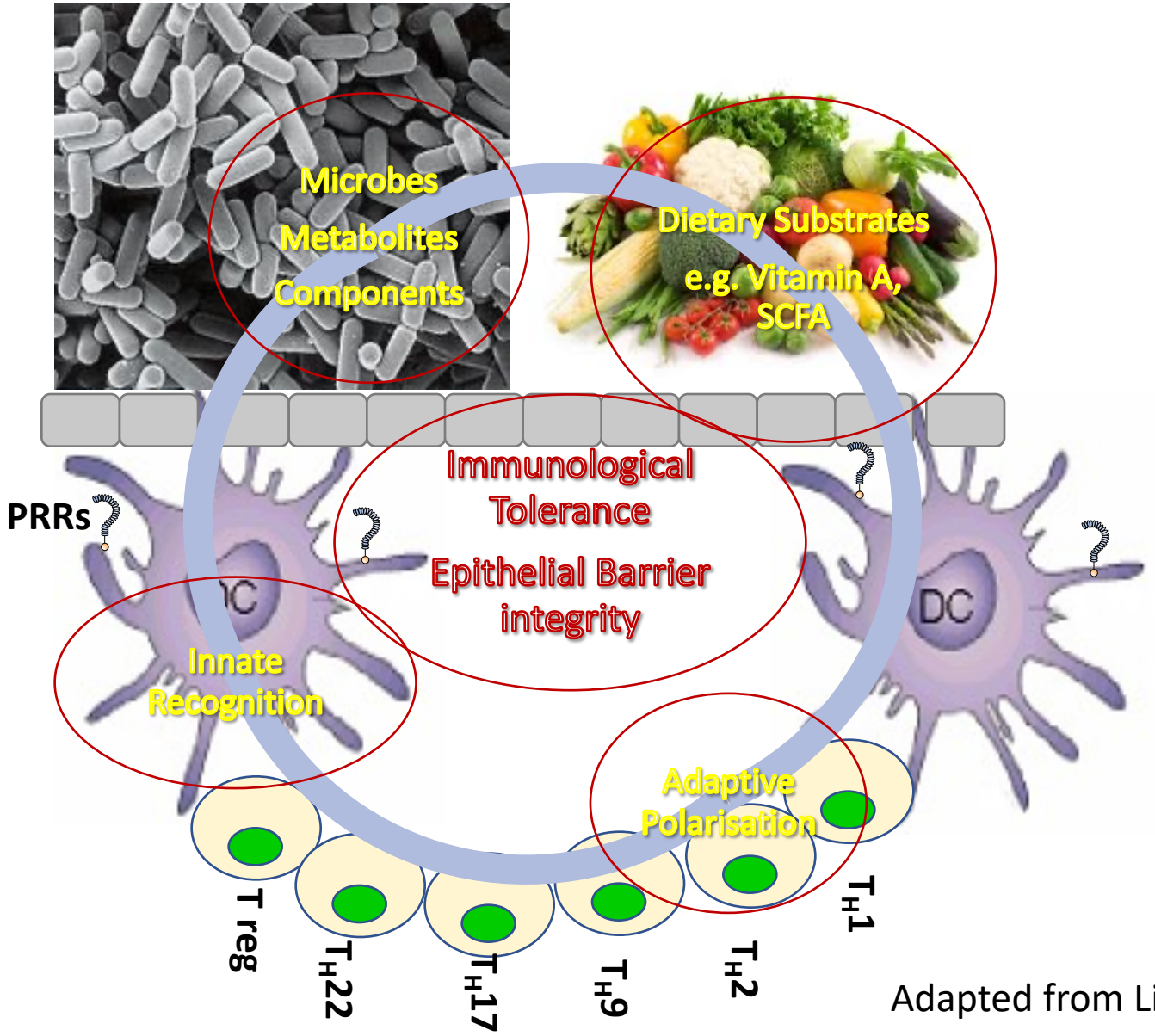


	Asthma
	OR* and 95% CI
Yogurt	
3-12mo	0.47 (0.26-0.84)
>12mo, ref	1
Other milk products	
3-12mo	0.37 (0.22-0.64)
>12mo, ref	1
Butter	
3-12mo	0.45 (0.26-0.77)
>12mo, ref	1

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

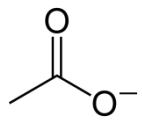
Roduit et al. JACI 2014

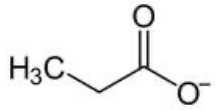
Regulation of Mucosal Inflammation through the interplay of microbiome and nutrition

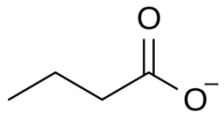


Adapted from Liam o'Mahony, Cork, Ireland

Short-chain fatty acids – Acetate, Propionate, Butyrate







-> Present in milk products

-> Produced by fermentation of undigested food – fiber/starch in the cecum and the proximal colon by the gut microbes

- substrate source
- colonic pH
- abundance and composition of the gut microbes
- gut transit time

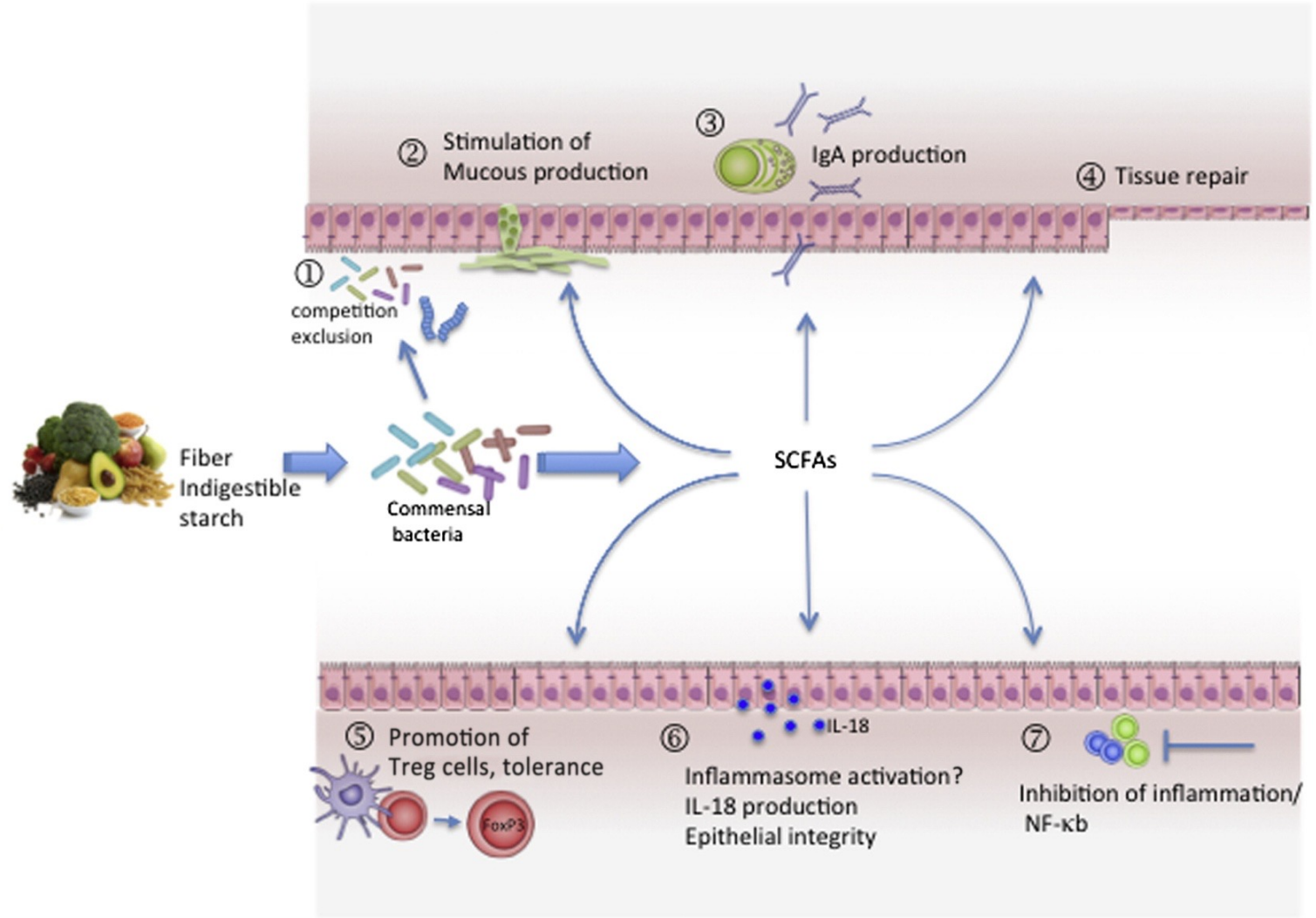
Butyrate: the main energy source of colonocytes
covers up to 10% of total human energy requirement

Acetate: transported to the liver as a substrate for cholesterol synthesis

Propionate: transported to the liver as a substrate for gluconeogenesis, lipogenesis, and protein synthesis

Van der Beek, et al., Nutrition Reviews, 2017

Mechanisms of gut homeostasis



mediated by GPCR and inhibition of histone deacetylase (HDAC) activity

Thorburn. et al Immunity 2014

Characterization of children with high levels of butyrate
Nutrition in the 1st year of life

TABLE 2 Association between early life exposures and fecal SCFA levels

Exposures	Butyrate				Propionate			
	GMR	95% CI		*P* value	GMR	95% CI		*P* value
Food introduced within 1st year								
Farm milk: yes vs. no	1.02	0.88	1.19	0.747	1.06	0.91	1.23	0.438
Cow's milk: yes vs. no	1.04	0.90	1.20	0.569	1.06	0.92	1.23	0.425
Yogurt: yes vs. no	**1.20**	**1.00**	**1.44**	**0.045**	1.09	0.91	1.31	0.362
Fish: yes vs. no	**1.21**	**1.05**	**1.40**	**0.010**	0.98	0.84	1.14	0.783
Nuts: yes vs. no	0.92	0.78	1.10	0.364	1.01	0.85	1.20	0.923
Vegetables or fruits (in first 6 mo): yes vs. no	**1.18**	**1.02**	**1.35**	**0.025**	0.98	0.85	1.14	0.804
Butter: yes vs. no	0.94	0.81	1.10	0.456	0.95	0.82	1.11	0.545
Margarine: yes vs. no	0.95	0.82	1.10	0.514	**0.85**	**0.74**	**0.99**	**0.031**
Chocolate: yes vs. no	0.99	0.86	1.14	0.895	**1.20**	**1.04**	**1.38**	**0.014**
Egg: yes vs. no	0.96	0.82	1.00	0.554	1.03	0.88	1.20	0.748
Cereals (in first 9 mo):								
yes vs. no	0.88	0.76	1.03	0.107	0.88	0.75	1.02	0.098
Meat (in first 9 mo): yes vs. no	1.13	0.96	1.32	0.136	0.98	0.83	1.15	0.778

Boldface values are significant (*P*-value < 0.05)

Roduit and Frei, Allergy 2019

Characterization of children with high levels of butyrate
Health outcomes

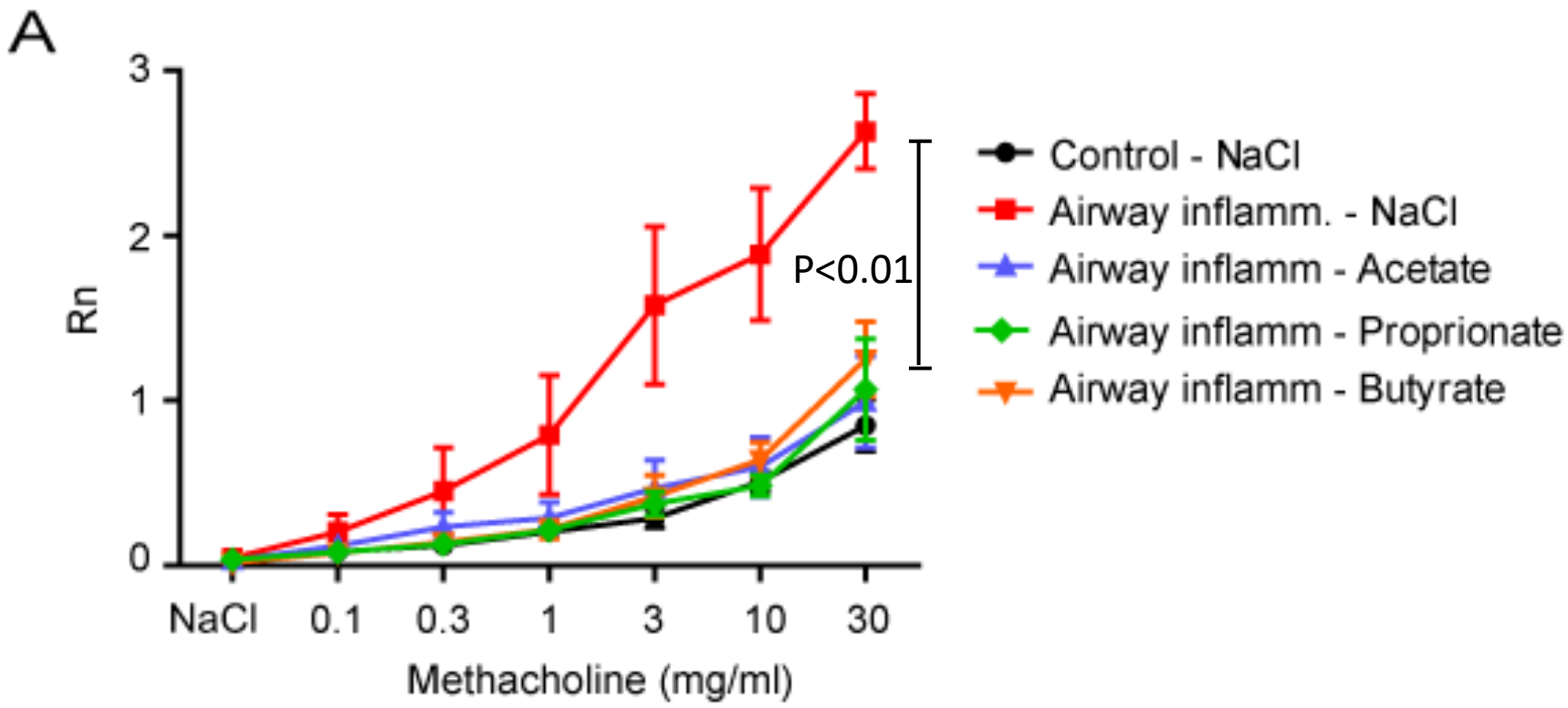
TABLE 3 Comparisons between children with high and low SCFA levels (≥95th percentile and <95th percentile, respectively)

	Butyrate <95P (<26.88 μmol/g)		Butyrate ≥95P (≥26.88 μmol/g)		Propionate <95P (<32.87 μmol/g)		Propionate ≥95P (≥32.87 μmol/g)	
	n	%	n	%	n	%	n	%
Asthma up to 6 yrs	32/262	12.2	1/15	6.7	32/262	12.2	1/15	6.7
Allergic rhinitis up to 6 yrs	27/252	9.7	0/15	0.0	25/279	9.0	2/15	13.3
Food allergy up to 6 yrs	32/275	11.6	1/15	6.7	30/275	10.9	3/15	20.0
Atopic dermatitis up to 6 yrs	135/284	47.5	5/16	31.3	133/285	46.7	7/15	46.7
Inhalant sensitization at 6 yrs	107/261	41.0	3/15	20.0	108/261	41.4	2/15	13.3
Food sensitization at 6 yrs	100/261	38.3	2/15	13.3	99/261	37.9	3/15	20.0
Any sensitization at 6 yrs	147/261	56.3	4/15	26.7	148/261	56.7	3/15	20.0

If the n per group was ≥3, a Fisher's exact test to compare two proportions was performed (*P*-value <0.05 was observed only with any sensitization at 6 yrs for butyrate and propionate).

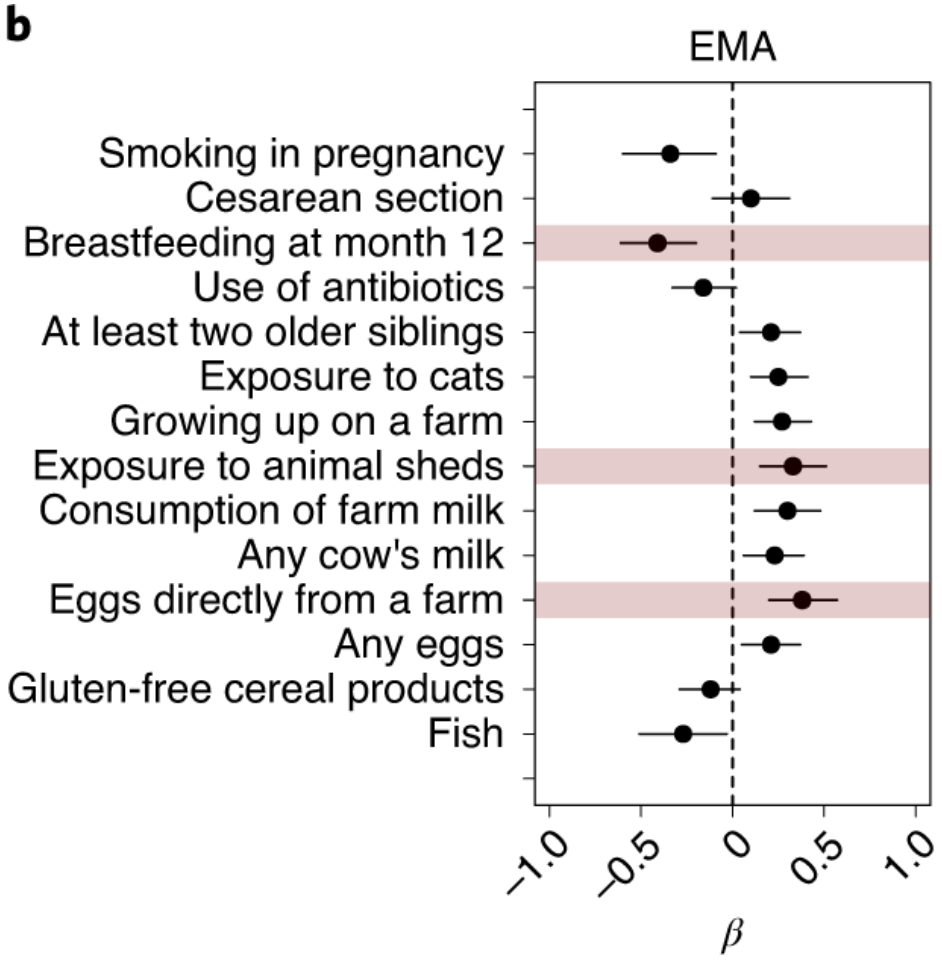
Roduit and Frei, Allergy 2019

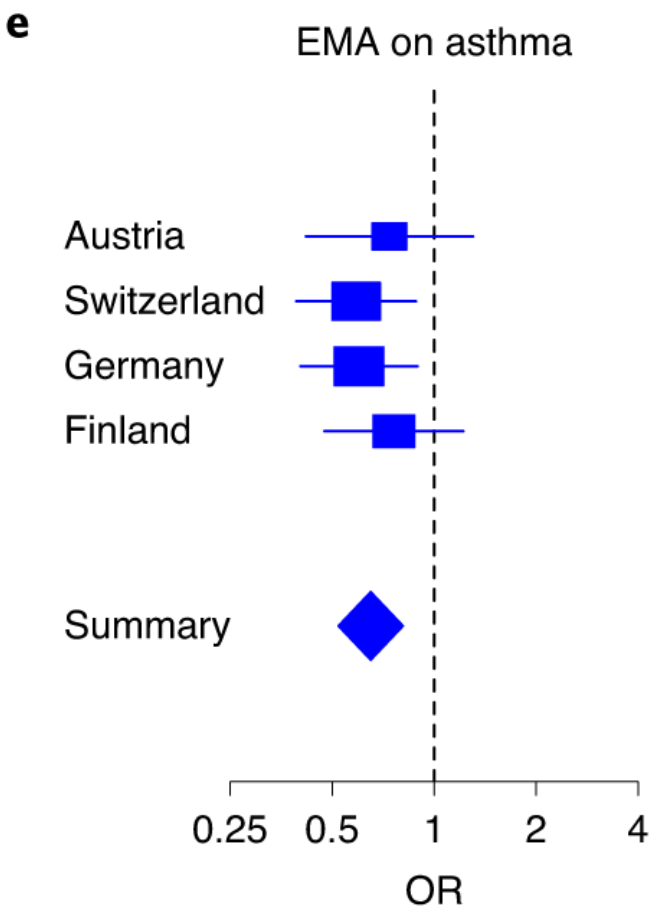
Oral application of SCFA to mice reduced the severity of airway inflammation



Roduit and Frei, Allergy 2019

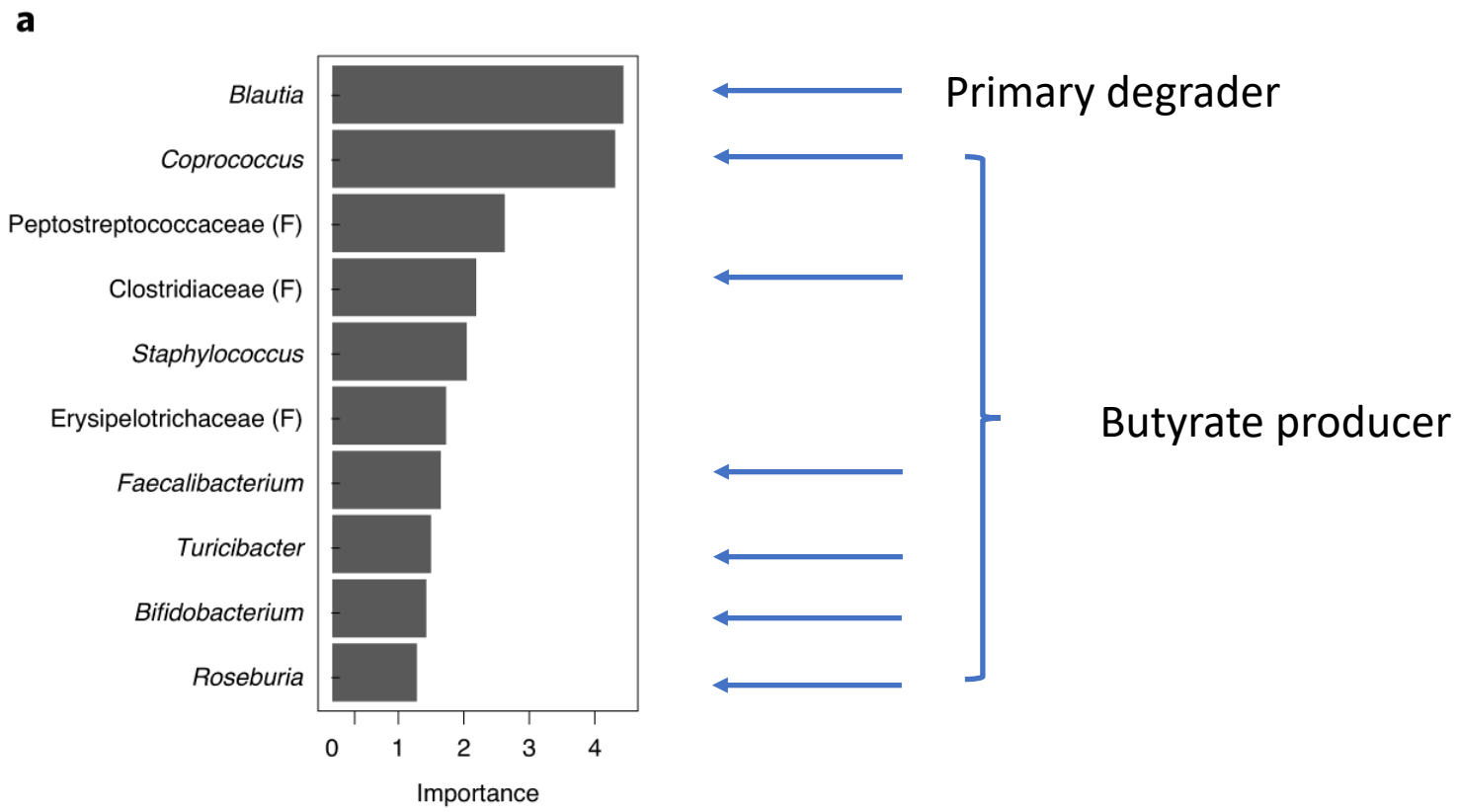
Farmers' children have a more 'mature' gut microbiome (EMA), which was associated with less asthma prevalence later in life





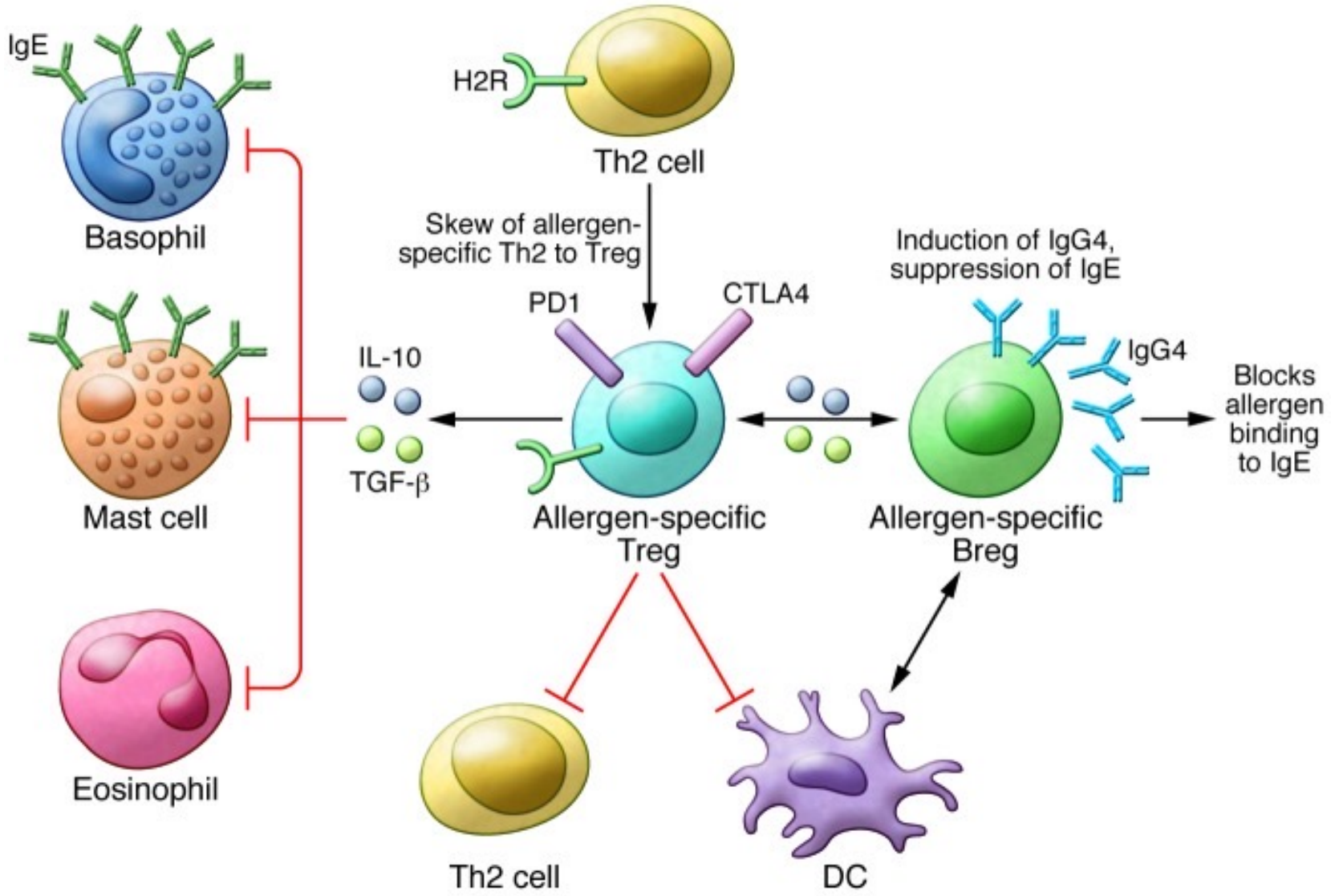
Depner et al., Nature Medicine 2020

A more ‘mature’ gut microbiome consists predominantly of butyrate-producing bacteria



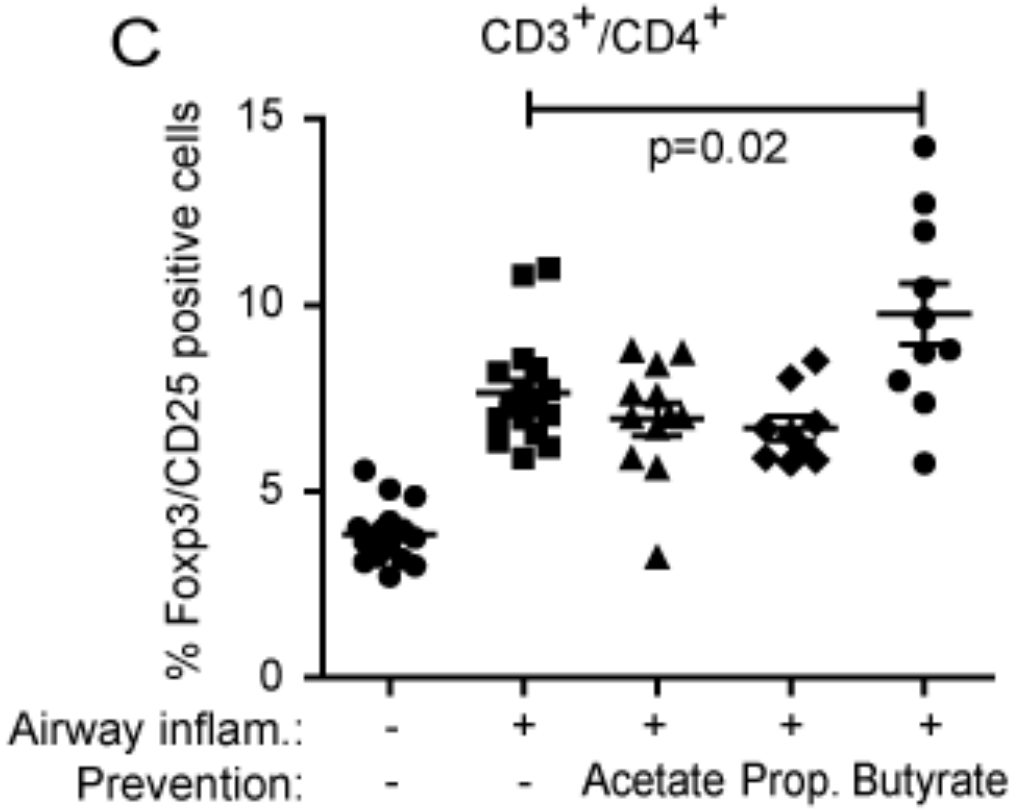
Depner et al., Nature Medicine 2020

Mechanisms of immune tolerance to allergens



Akdis and Akdis, J Clin Invest. 2014

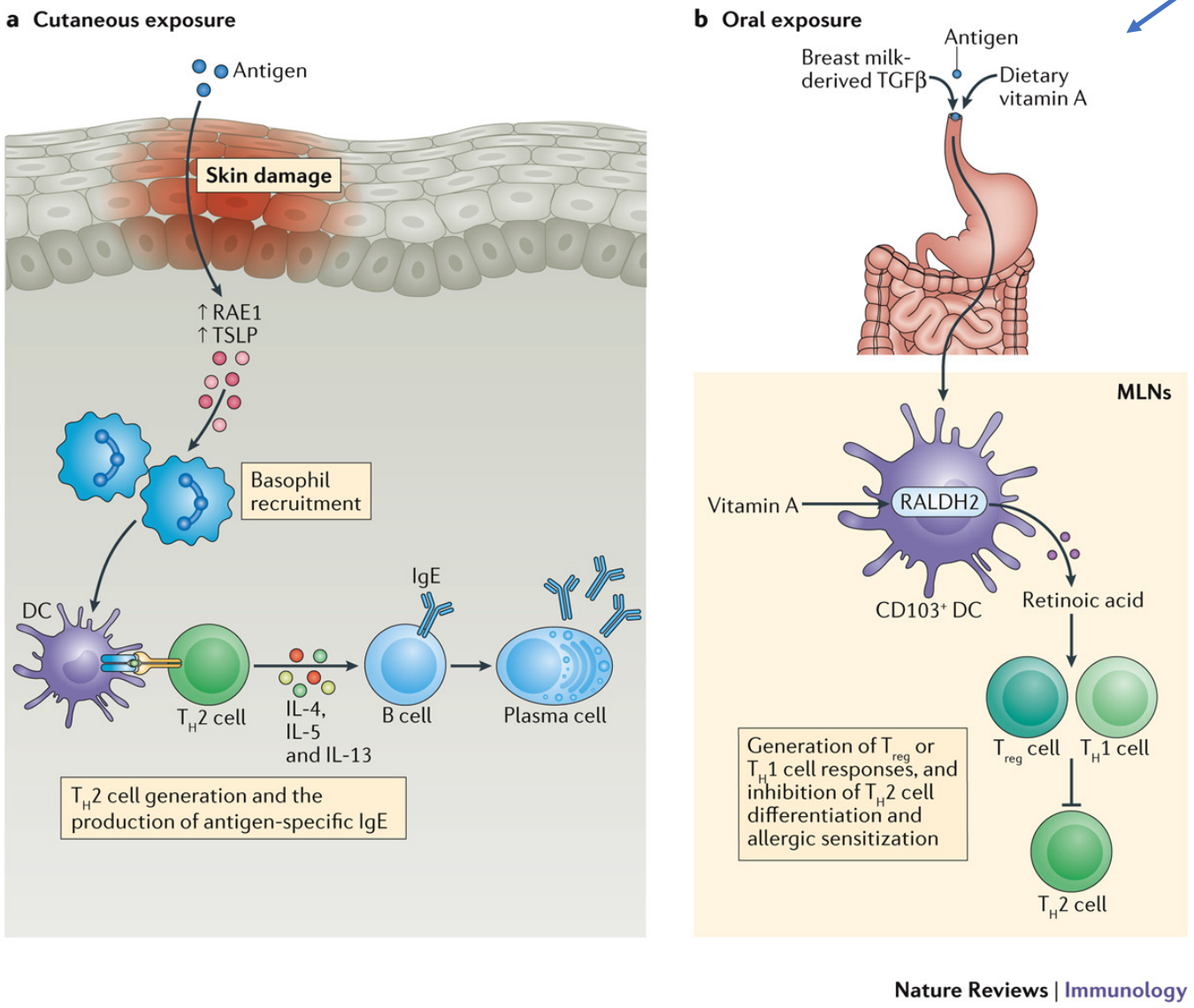
Oral application of Butyrate induced regulatory T cells in mice



Roduit and Frei, Allergy 2019

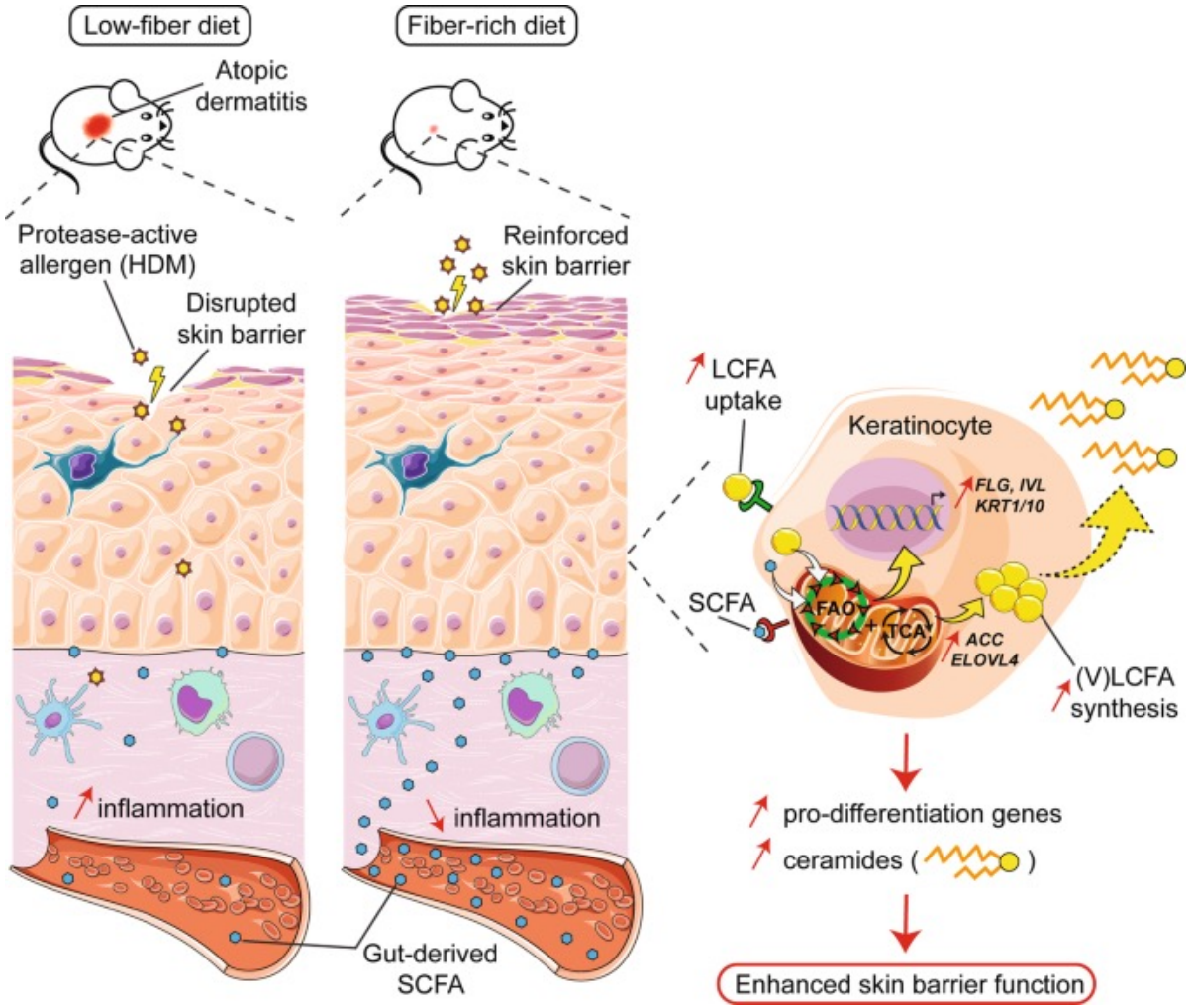
Immunological tolerance

Fiber



Reynolds and Finlay, Nature reviews Immunology 2017

Gut-derived short-chain fatty acids modulate skin barrier integrity by promoting keratinocyte metabolism and differentiation

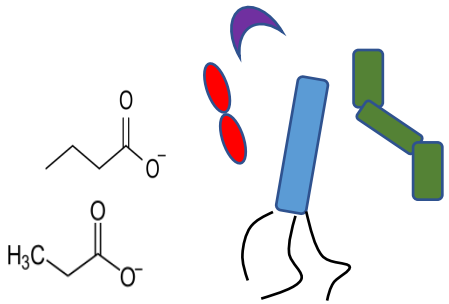


Trompette et al. Mucosal Immunol. 2022

CARE birth cohort study

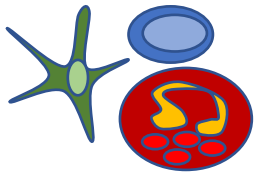
Early-life nutrition
Weekly diary
Mother milk composition

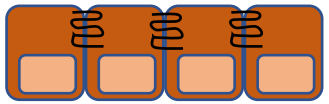
Environment
Questionnaires



Gut and Skin
Microbiome
Metabolites
Endospores

Immune system
Tolerance





Skin
Barrier integrity

Prevention of the development allergic diseases

Childhood **A**lle**R**gy, Nutrition and **E**nvironment

CARE-Studie

- Prospective birth cohort study on allergy prevention

Hypothesis:

- Nutrition, especially in the first year of life, could influence the development of allergic diseases
- Increased diet diversity in early life is protective against allergies

Aim:

- To identify and analyze foods or food components which could be used for new prevention strategies in allergy

CARE - Study design

- **Recruitment: N**ewborns at the Kantonspital of St. Gallen
- **Follow-up:** at least 5 years
- **Primary outcome:** Atopic dermatitis
- **Secondary outcomes:** Food allergy, sensitization and other allergic diseases.
- **Exclusion criteria: M**other having a history of any auto-immune disease, or the child having any congenital abnormalities, chronic lung disease or immune deficiency.

Methods

Nested case-control study based on the availability of fecal samples and questionnaire responses
66 children

- **39 children with no history of atopic dermatitis (AD) (non-AD group)**
- **27 children with AD (AD group)**

in the first year of life

AD within the first year of life was defined as having either,
a) a doctor's diagnosis of AD or
b) itchy rash at specific locations reported by the parents in the 4 months or 1 year old questionnaire, or
c) AD diagnosed by the research doctor at the 4 months old or 1-year old physical examination.

Fecal samples

- 90 days (mean +/- SD, 94+/-17 days) n= 62
- 180 days (181+/-14 days) n=66
- 360 days (362+/-18 days) n=66

➢ 16S gene amplicon sequencing (Phylum and Family level), qPCR, and HPLC

Sasaki et al., Allergy, 2022

Association Between Atopic Dermatitis Phenotypes and Other Allergic Diseases up to 6 Years of Age

Variable	No./Total No. (%)	OR (95% CI)	OR[a] (95% CI)
Asthma	78/923 (8.5)	NA	NA
Early transient	10/86 (11.6)	1.62 (0.79-3.315)	1.60 (0.77-3.305)
Early persistent	10/57 (17.5)	2.62 (1.26-5.475)	2.87 (1.31-6.315)
Late	3/48 (6.3)	0.82 (0.25-2.735)	0.83 (0.25-2.805)
Never/infrequent	55/732 (7.5)	1 [Reference]	1 [Reference]
Food allergy	78/864 (9.0)	NA	NA
Early transient	16/80 (20.0)	3.8 (2.02-7.13)	3.69 (1.93-7.035)
Early persistent	19/56 (33.9)	7.8 (4.13-14.72)	7.08 (3.59-13.975)
Late	1/48 (2.1)	0.32 (0.04-2.4)	0.32 (0.04-2.395)
Never/infrequent	42/680 (6.2)	1 [Reference]	1 [Reference]
Allergic rhinitis	73/921 (7.9)	NA	NA
Early transient	9/86 (10.5)	1.82 (0.86-3.88)	1.90 (0.88-4.115)
Early persistent	12/57 (21.0)	4.16 (2.05-8.42)	4.04 (1.82-8.955)
Late	8/48 (16.7)	3.12 (1.38-7.07)	3.23 (1.37-7.615)
Never/infrequent	44/730 (6.0)	1 [Reference]	1 [Reference]

Abbreviations: NA, not applicable; OR, odds ratio.

[a] Adjusted for farmer, center, sex, and breastfeeding.

Roduit et al. JAMA Pediatrics 2017

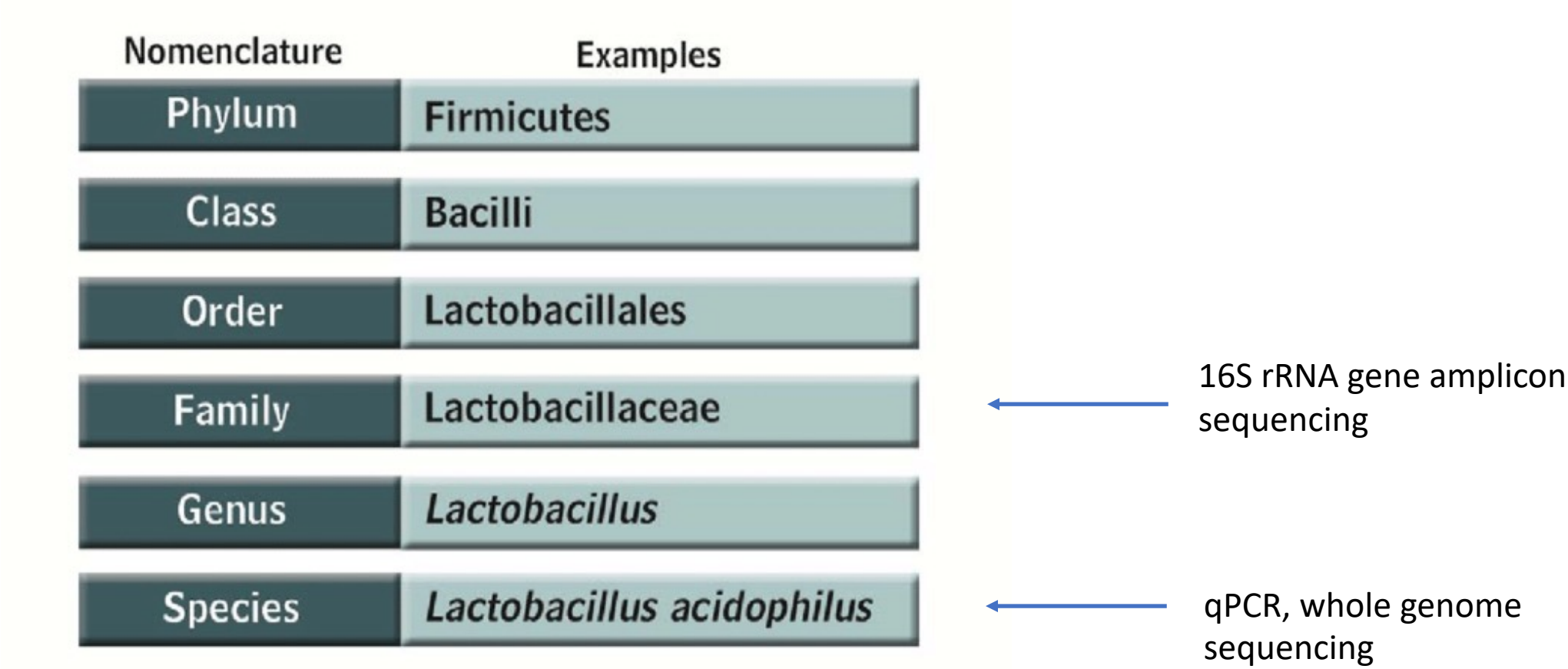
Aims and objective

- Longitudinal comparison of the gut microbiota in children with or without atopic dermatitis

- Abundance of butyrate producers, key primary degraders, and butyrate in children with or without atopic dermatitis

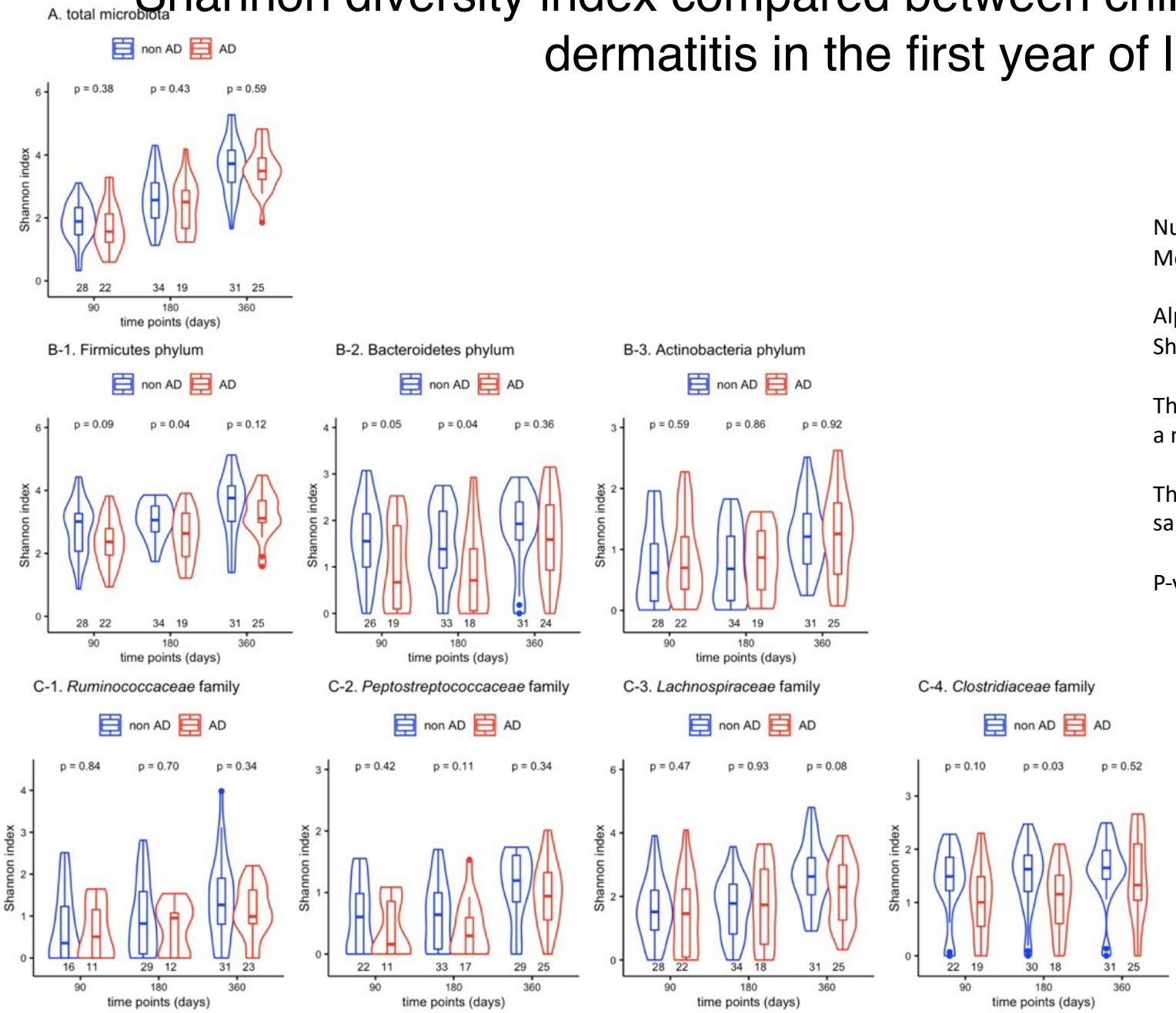
Sasaki et al., Allergy, 2022

CARE-Study – Bacteria taxonomy



Simrén et al. Rome Foundation Report – Intestinal Microbiota in Functional Bowel Disorders 2012

Shannon diversity index compared between children with and without atopic dermatitis in the first year of life for the



Number of samples: n=50 (90 days), 53 (180 days) and 56 (360 days)
Method: 16S rRNA gene sequencing

Alpha-diversity (within-sample diversity) of the total microbiota, based on the Shannon index

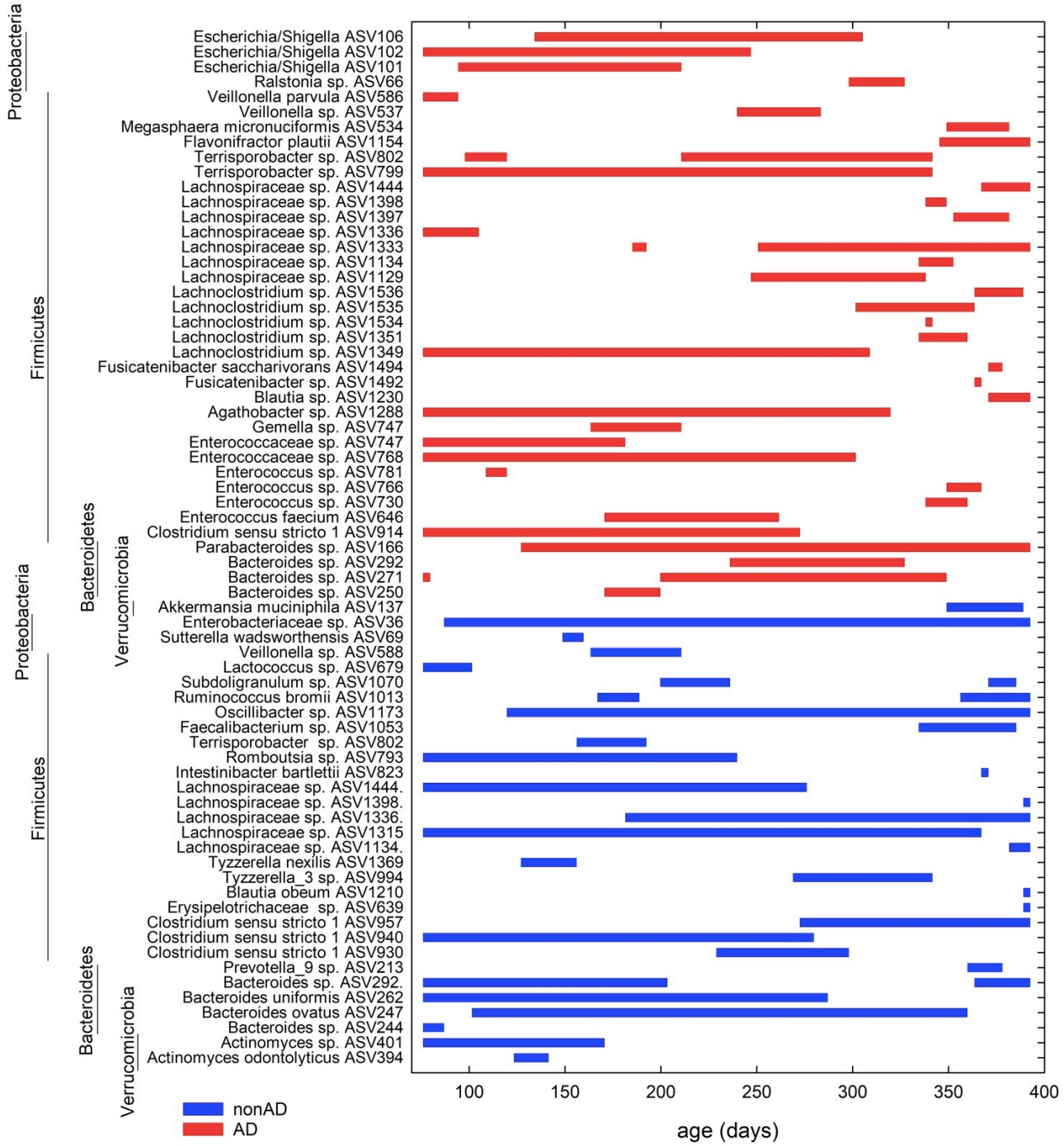
The violin plots describe the abundance with rotated kernel density plots, a marker and a box each indicating the median and interquartile range.

The number on the x-axis below each box plot describes the number of samples with species of the relevant taxa.

P-values are derived by Mann-Whitney U test.

Sasaki et al., Allergy, 2022

Time intervals of differentially abundant ASVs identified by MetaLonDa using the age of the samples in days.



Number of samples: n=168
Method: 16S rRNA gene sequencing including ASV with more than 50 reads

The blue lines represent the intervals where samples from children without atopic dermatitis (non-AD group) has more reads

The red lines represent those where samples from children with atopic dermatitis (AD group) has more reads.

ASV refers to single DNA sequences that are equivalent of strains

MetalonDa was used to assess differentially abundant ASV using a longitudinal method

Sasaki et al., Allergy, 2022

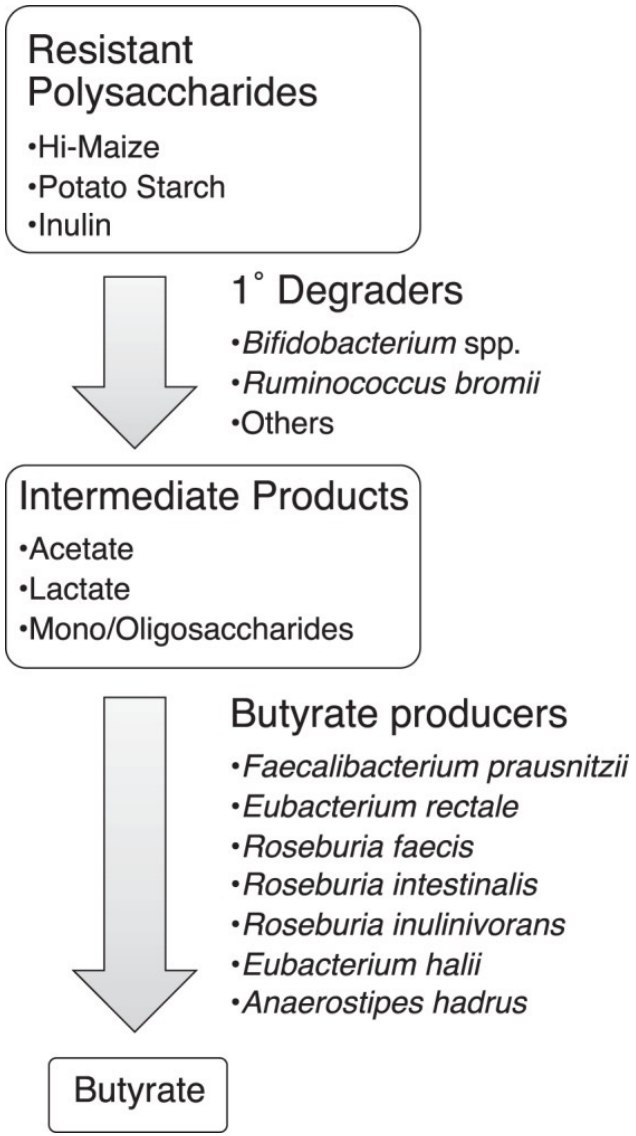
Aims and objective

- Longitudinal comparison of the gut microbiota and atopic dermatitis

- Abundance of butyrate producers, key primary degraders, and butyrate in children with or without atopic dermatitis

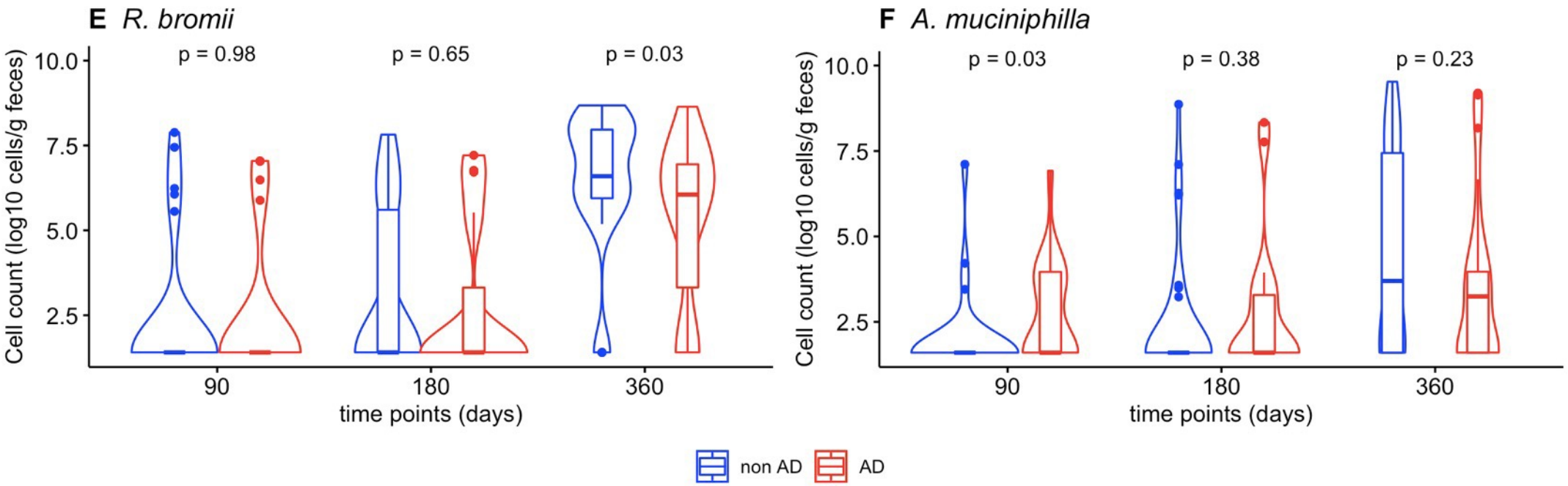
Sasaki et al., Allergy, 2022

Model of metabolites and microbes that catalyze the flow of carbon from resistant polysaccharides to butyrate



Baxter et al. mBio 2019

Abundance of major primary degraders among children with and without atopic dermatitis during their first year of life

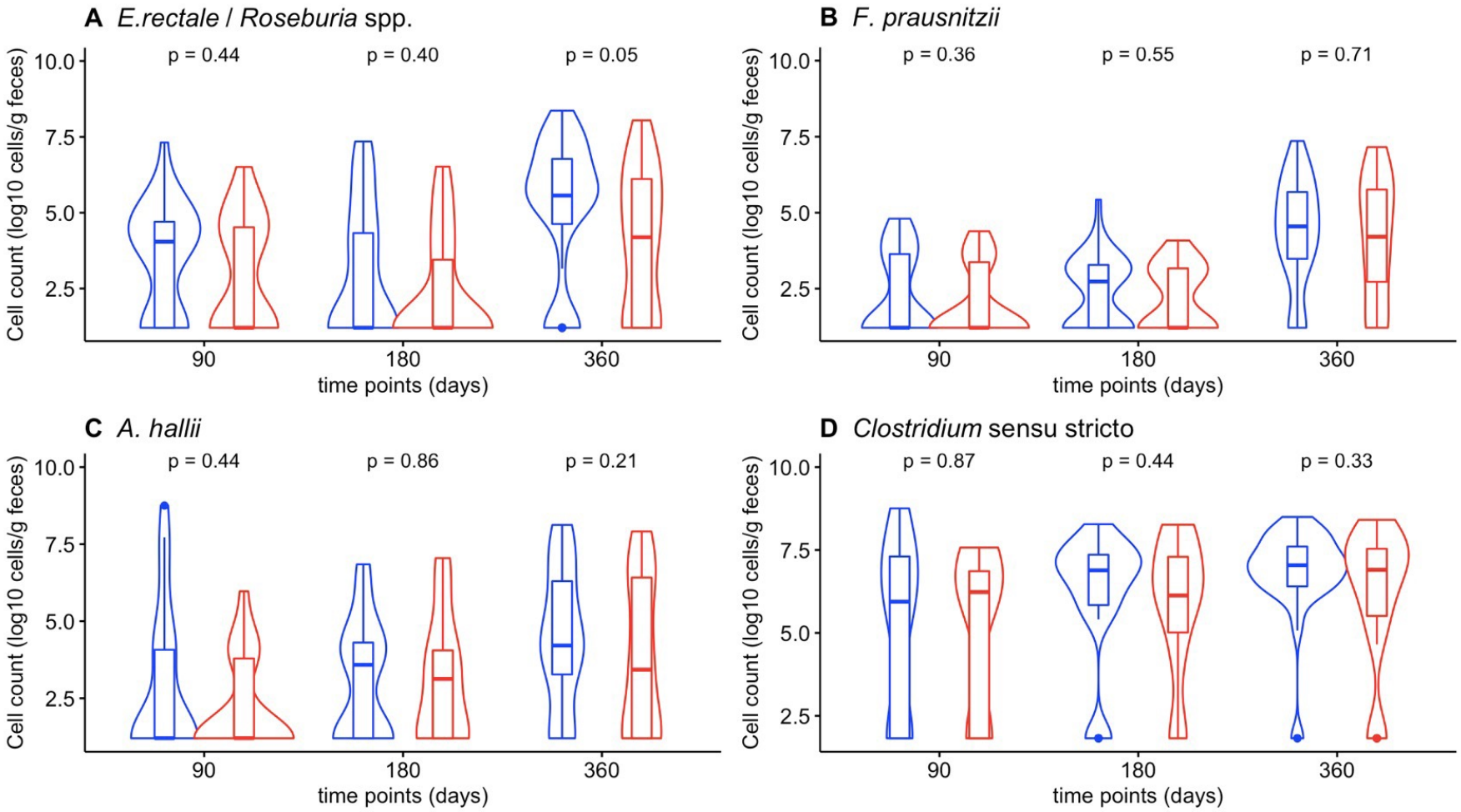


Number of samples: n=48 (90 days), 54 (180 days) and 66 (360 days)
Method: qPCR

The violin plots describe the abundance with rotated kernel density plots, a marker and a box each indicating the median and interquartile range. P-values are derived by Mann-Whitney U test.

Sasaki et al., Allergy, 2022

Abundance of major butyrate producing groups among children with and without atopic dermatitis during their first year of life

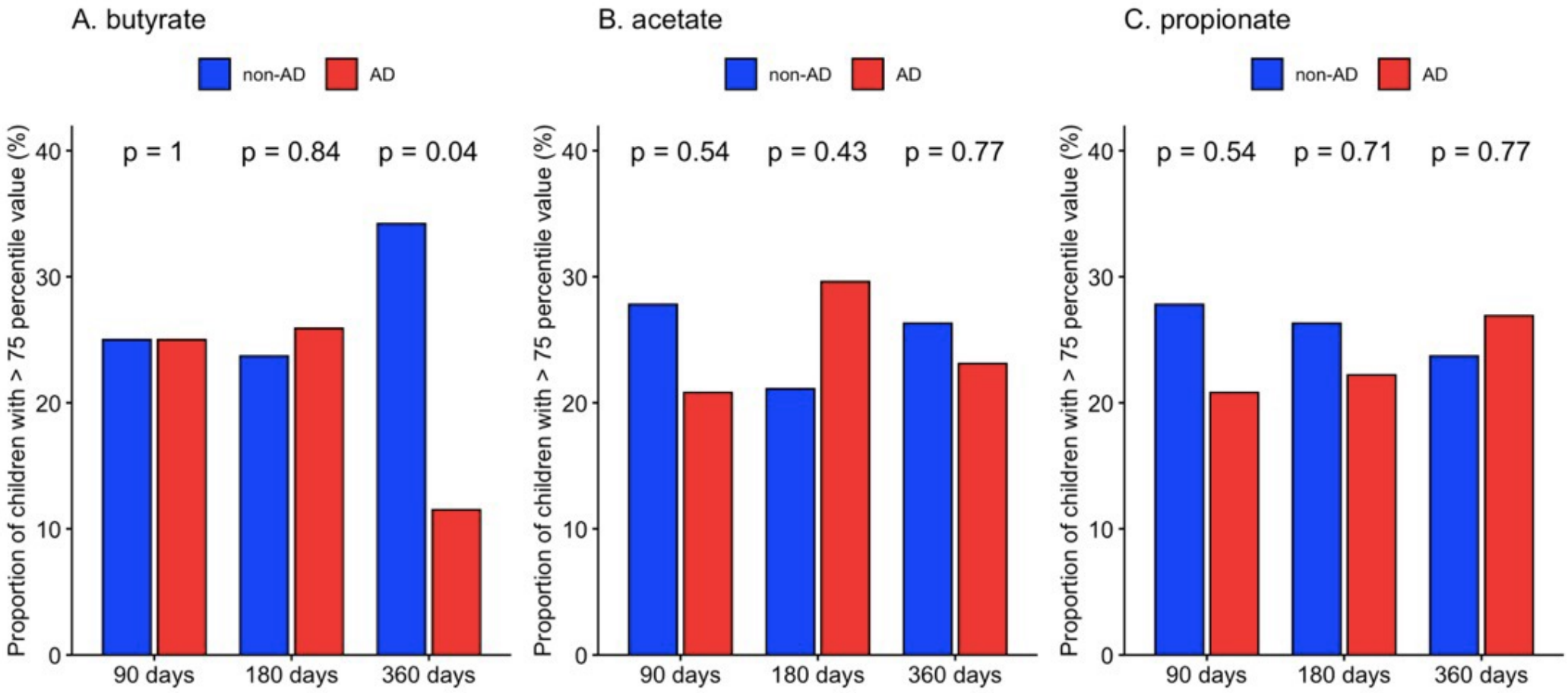


Number of samples: n=48 (90 days), 54 (180 days) and 66 (360 days)
Method: qPCR

Sasaki et al., Allergy, 2022

The violin plots describe the abundance with rotated kernel density plots, a marker and a box each indicating the median and interquartile range. P-values are derived by Mann-Whitney U test.

Association between the abundance of the three major short chain fatty acids among children with and without atopic dermatitis during their first year of life



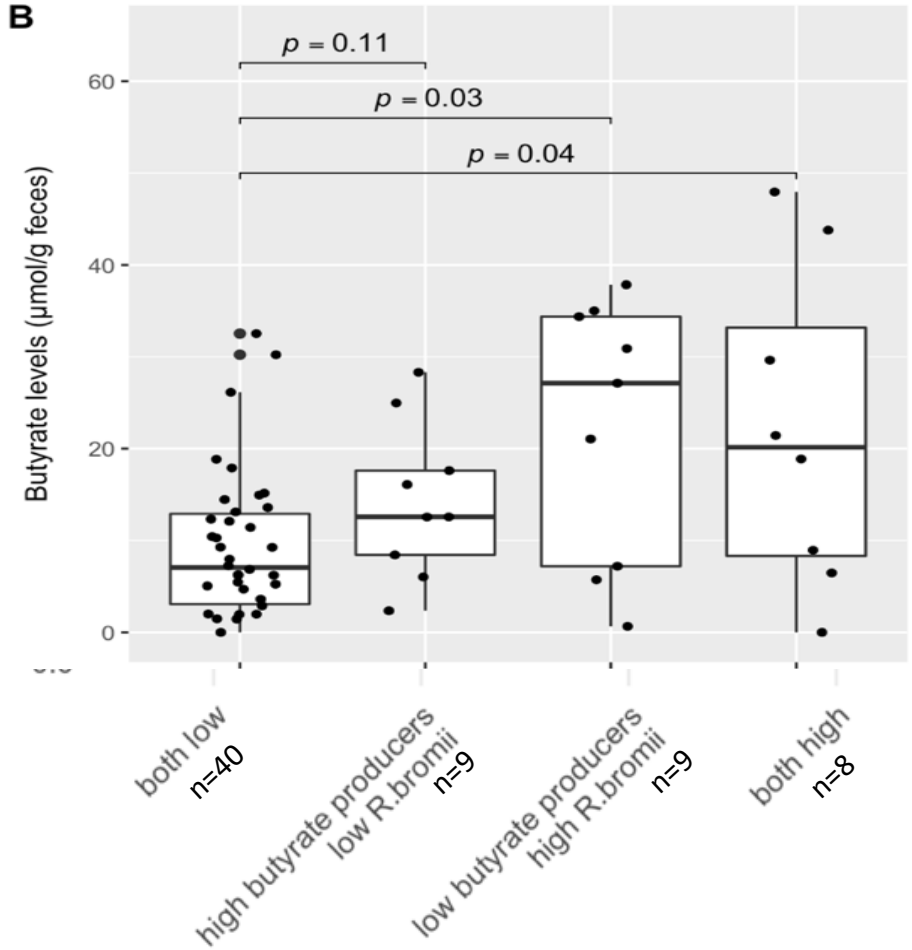
Number of samples n=60 (90 days), 65 (180 days), 64 (360 days)

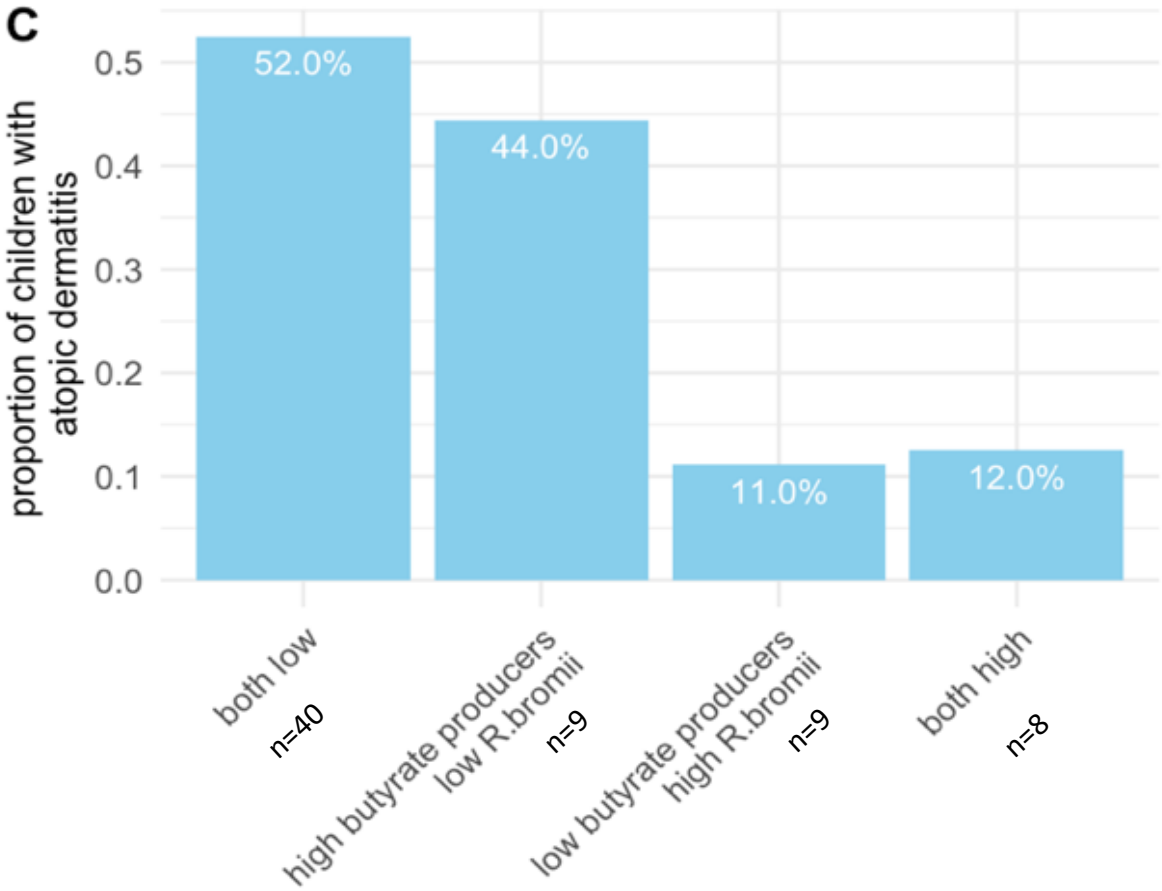
The proportion of children with high level of each butyrate, acetate and propionate was compared between the two groups using the 75-percentile value.

Method: HPLC; P-values were derived by Chi-squared test.

Sasaki et al., Allergy, 2022

Association between butyrate level, abundance of the major butyrate producers (Clostridium sensu stricto, E. rectale, A. hallii and F. prausnitzii), R. bromii at 360 days and AD during the first year of life.

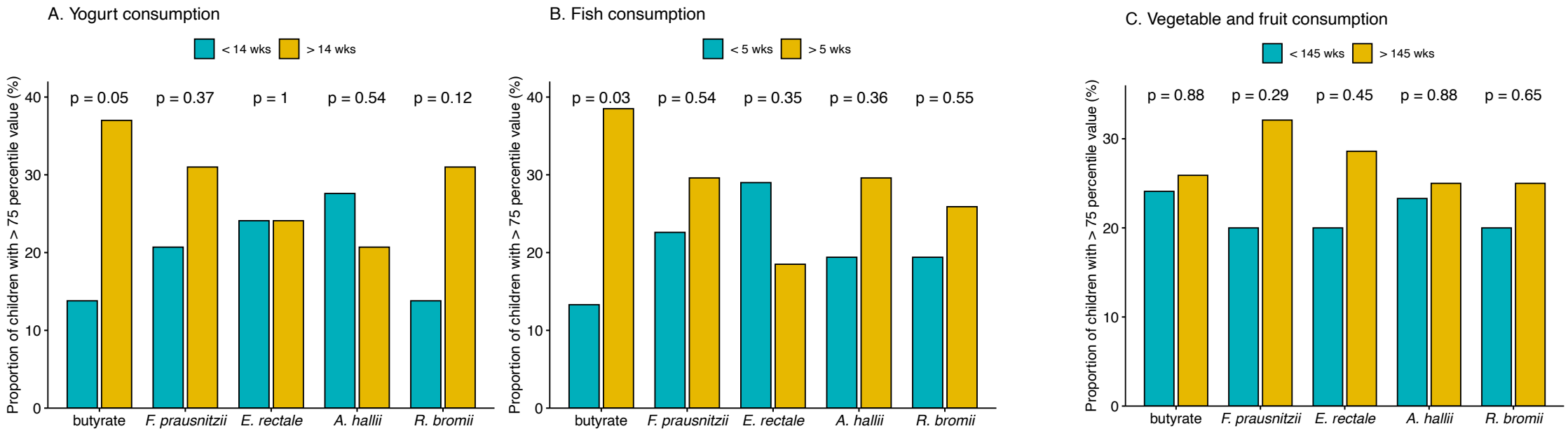




Categorisation using the 75-percentile value of the abundance of the sum of the 3 butyrate producers and R. bromii (7.06 and 7.71 log10 cells/g feces, respectively) as a cutoff
*p-values for B) were derived by Dunn test with Benjamini-Hochberg adjustment.

Sasaki et al., Allergy, 2022

Duration of consumption of selected food items during the first year of life and butyrate, abundance of major butyrate producers (*F. Prausnitzii*, *E. rectale* and *A. hallii*) and *R. bromii* at 360 days



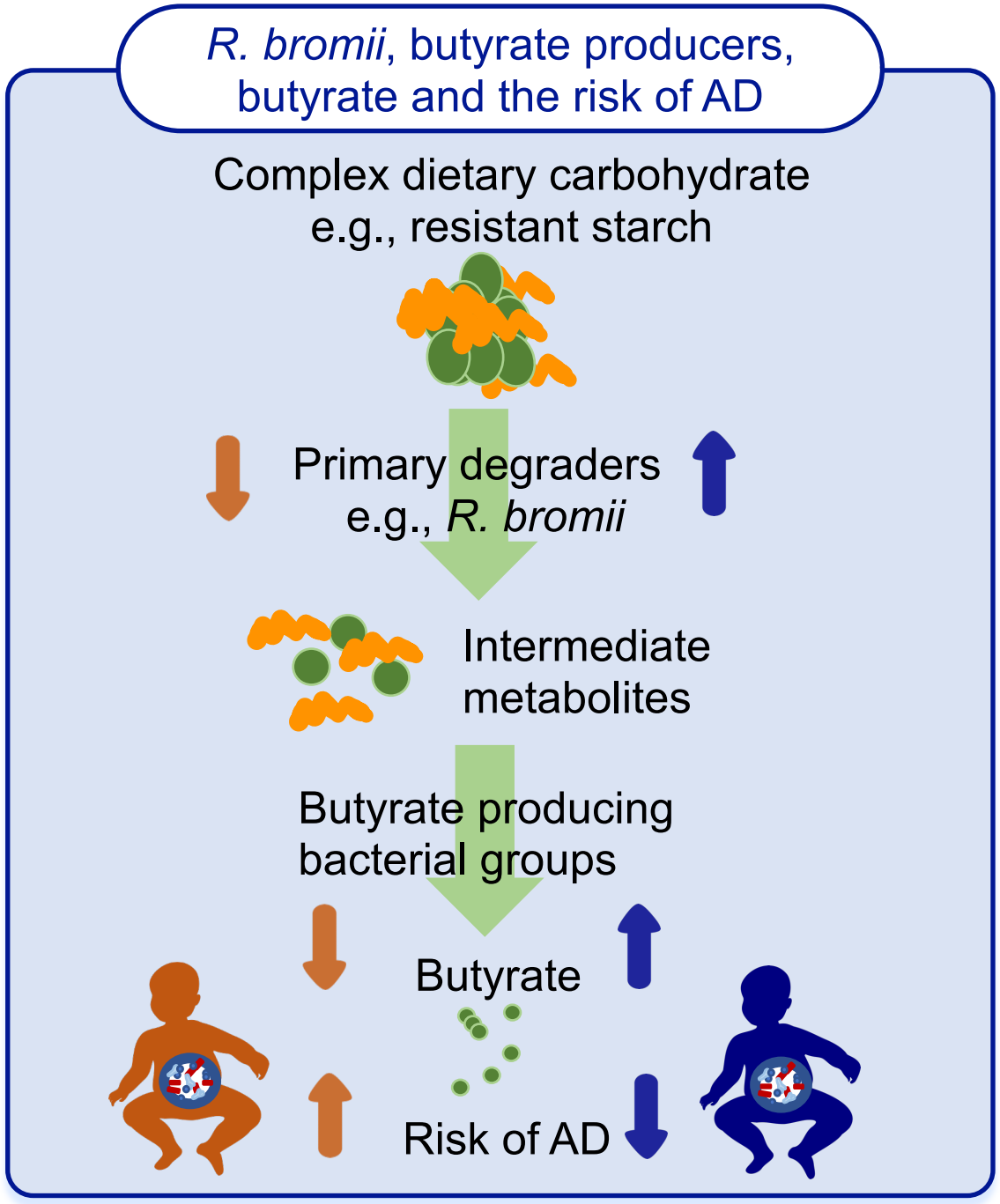
Duration of A. yogurt and B. fish consumption were each categorized at the median value, 14 weeks and 5 weeks, respectively. Duration of C. vegetable and fruit consumption was calculated as the sum of the duration of the 6 categories in the questionnaire and categorized at the median value of 14.5 weeks. For each food item, the proportion of children with high level of butyrate or abundance of selected bacterial groups using the 75-percentile value as a cut-off were compared between children who had consumed the food less and more than the median. P-values were derived by Chi-squared test.

Sasaki et al., Allergy, 2022

Conclusion

- Diversity (alpha-diversity) within the Firmicutes and Bacteroidetes phyla was lower in the AD group.

- Multiple species within the same bacterial family were differentially abundant at various timings and durations. This suggests that the key taxa characterising the microbiota of non-AD or AD children are at the lower taxonomic level.



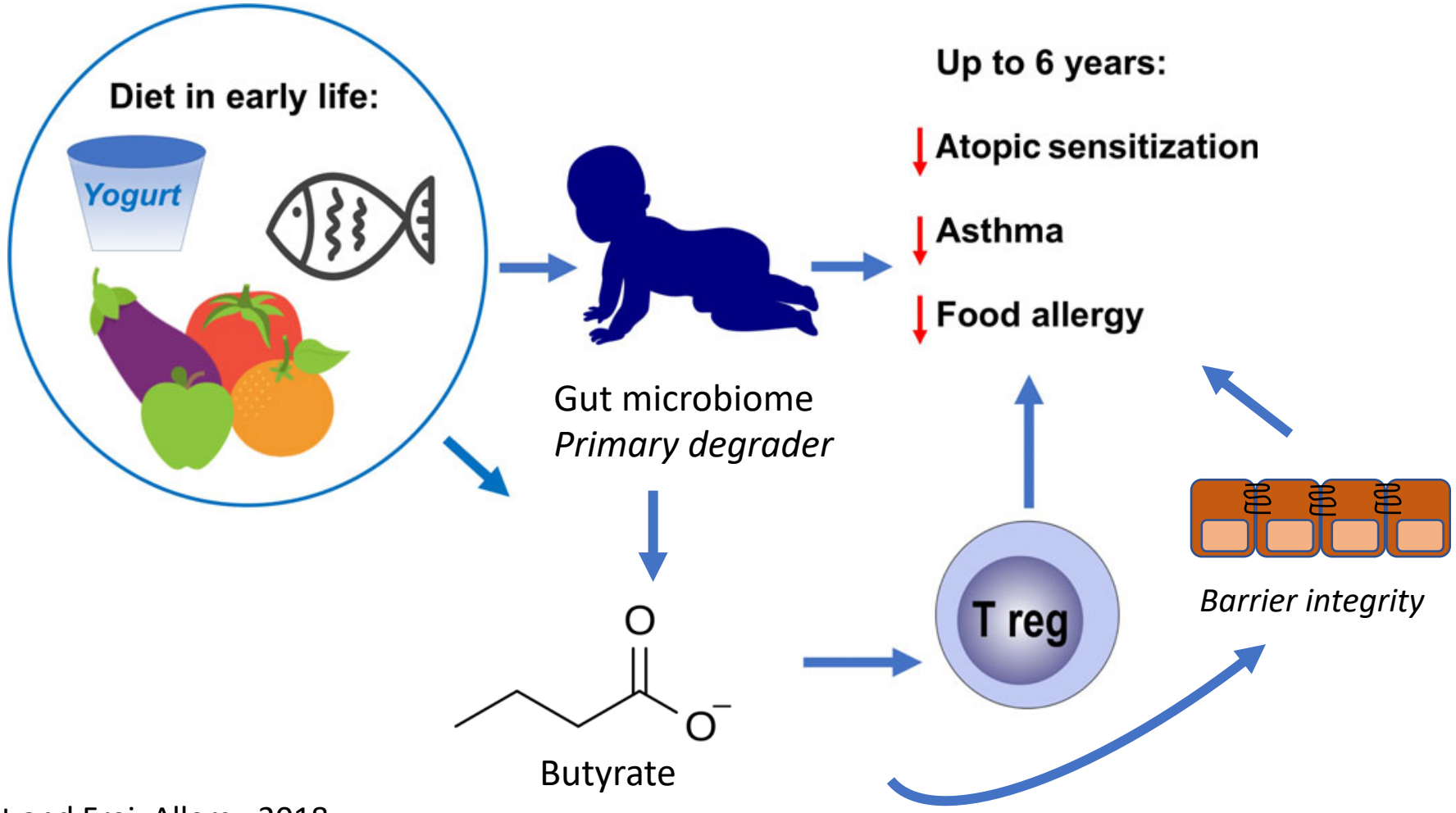
Conclusion

- Children with an increased abundance of a primary degrader - *R. bromii*
- Accompanied enhanced metabolic activity – butyrate

➢ Strongly reduced proportion of atopic dermatitis

➢ One potential mechanistic explanation for the association between the gut microbiome development and the risk to develop atopic dermatitis

Dietary SCFA levels influence the development of allergic diseases later in life



Adapted from Roduit and Frei, Allergy 2018

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