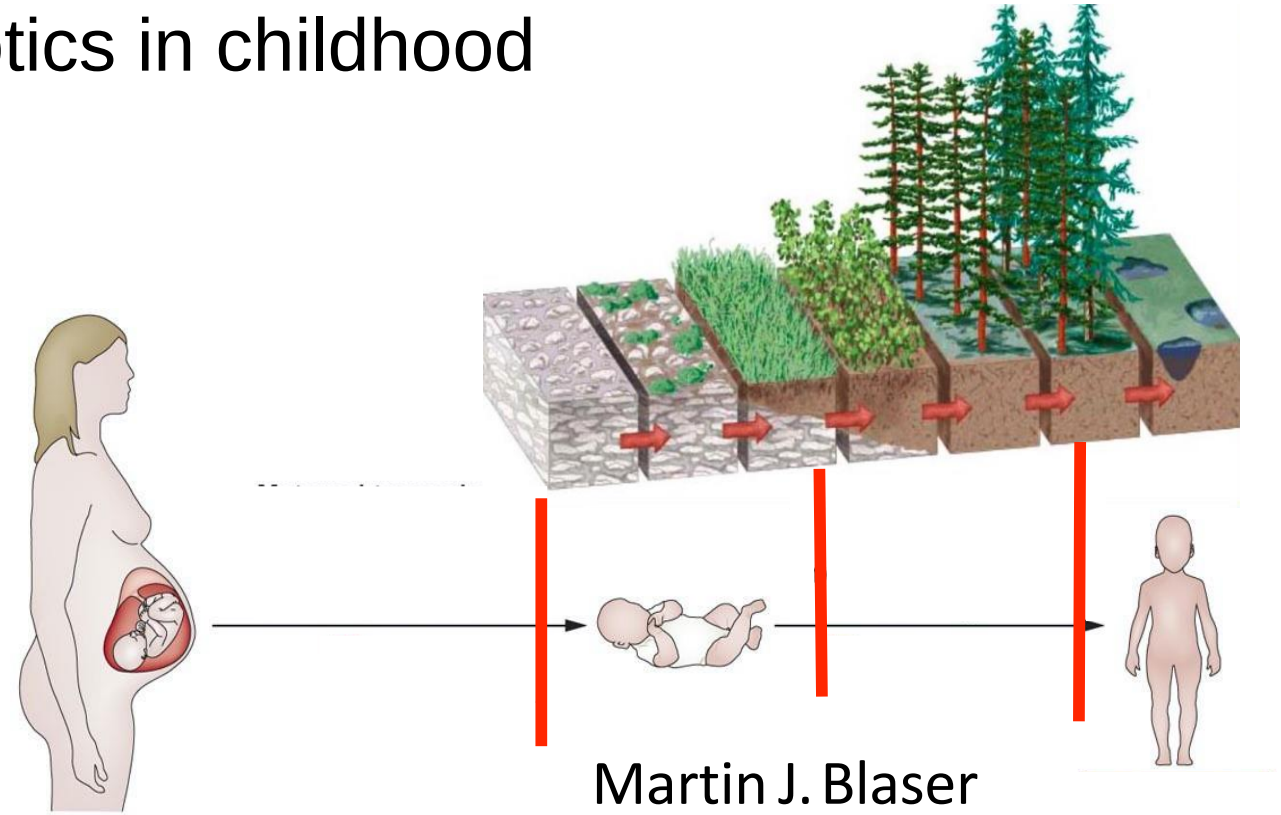
The unintended consequences of the use of antibiotics in childhood



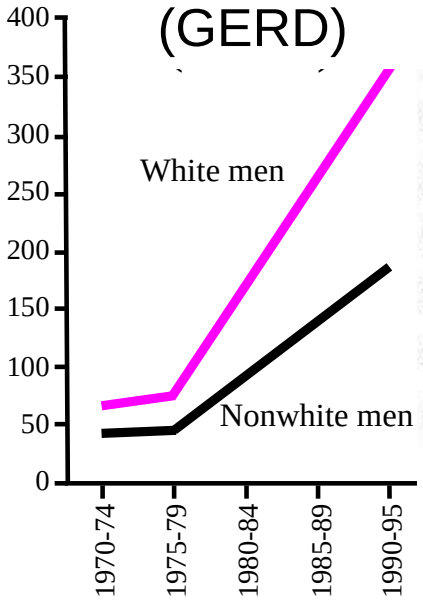
Martin J. Blaser
Center for Advanced Biotechnology and Medicine
Rutgers University, New Brunswick NJ

RWJ Department of Pediatrics
4 November 2023

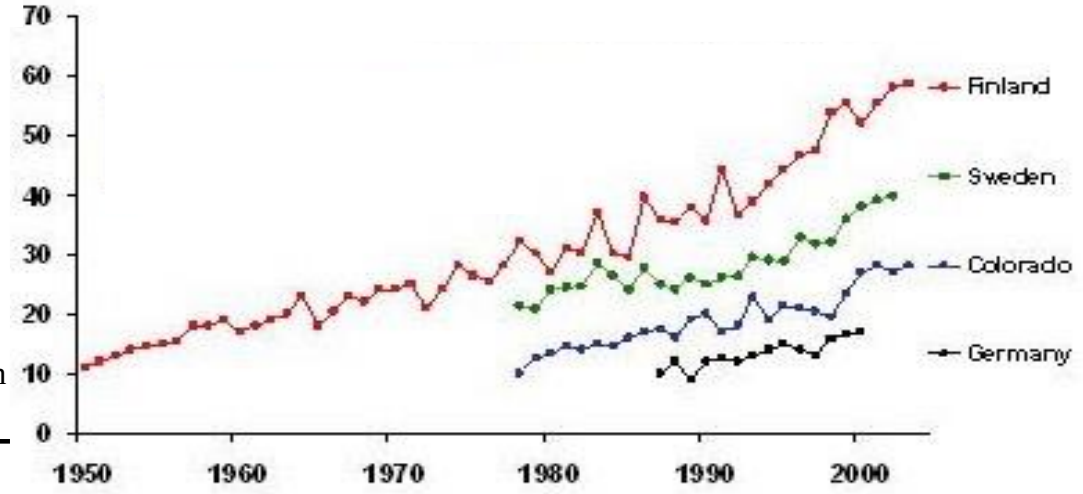
RUTGERS
Robert Wood Johnson Medical School

Why have many diseases increased in recent decades?

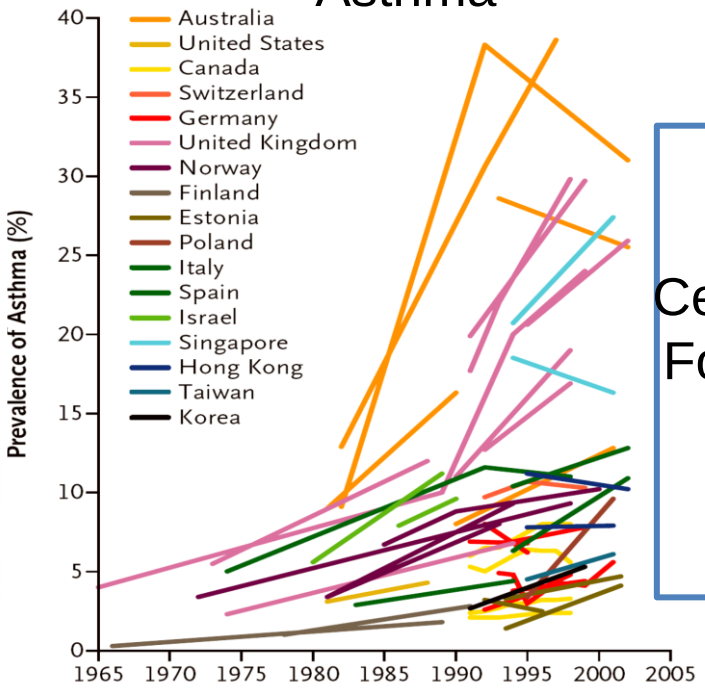
Reflux Disease (GERD)



Juvenile (type 1) diabetes



Asthma

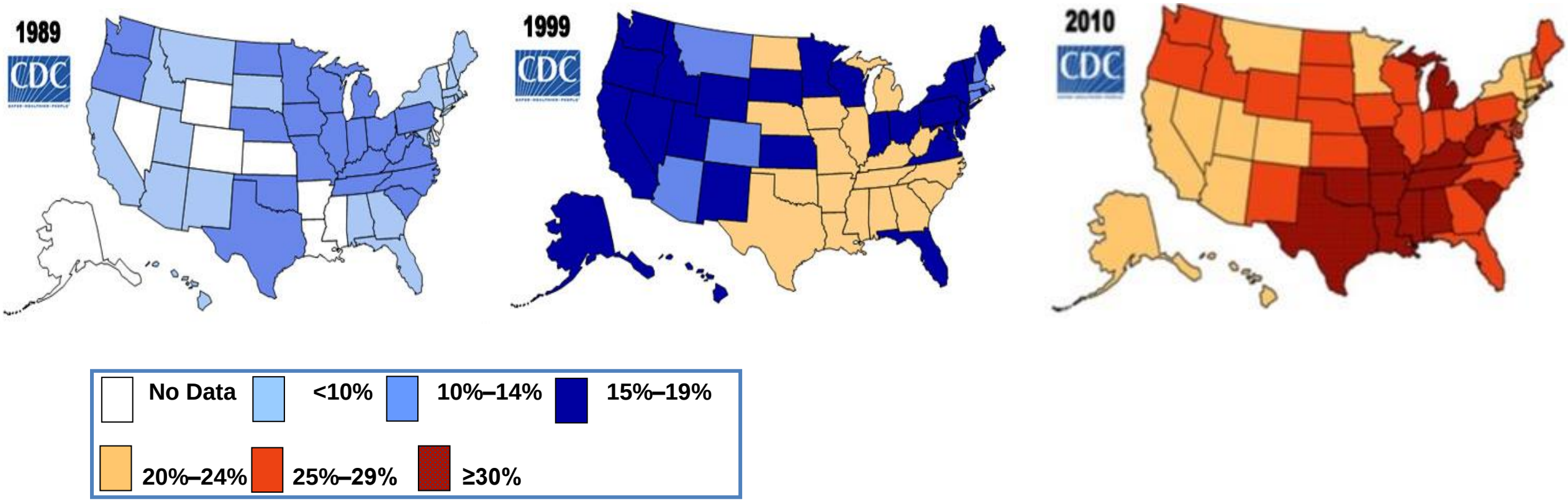


Obesity
IBD
Celiac Disease
Food allergies
Atopy
ADHD
Autism

Ann NY Acad Sci 2008 12:1150 *N Engl J Med* 2006;355:2226 *Gut* 1997;41:594

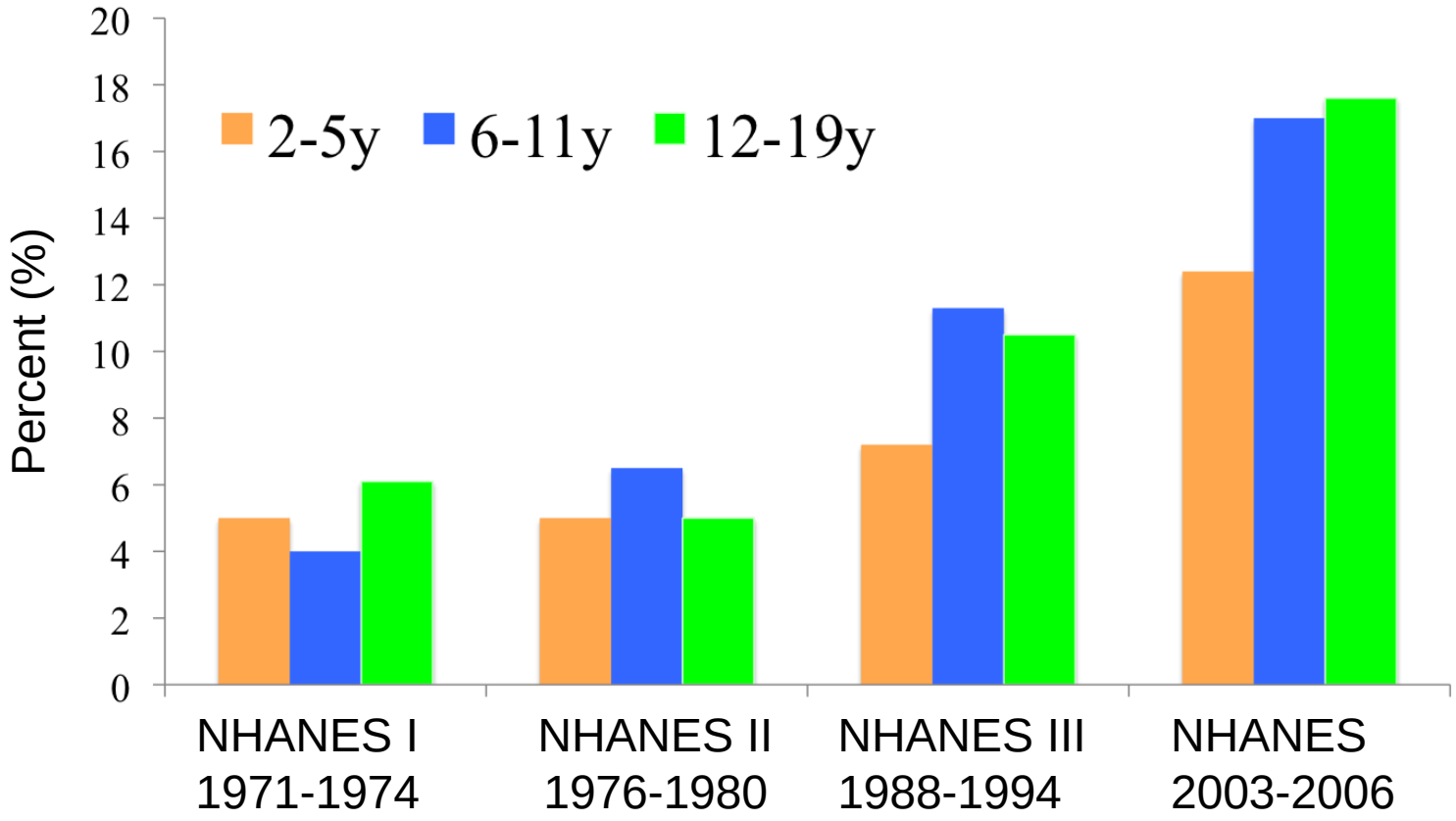
10 causes or a single cause?

Obesity trends in adults in 50 US states: changing physiology



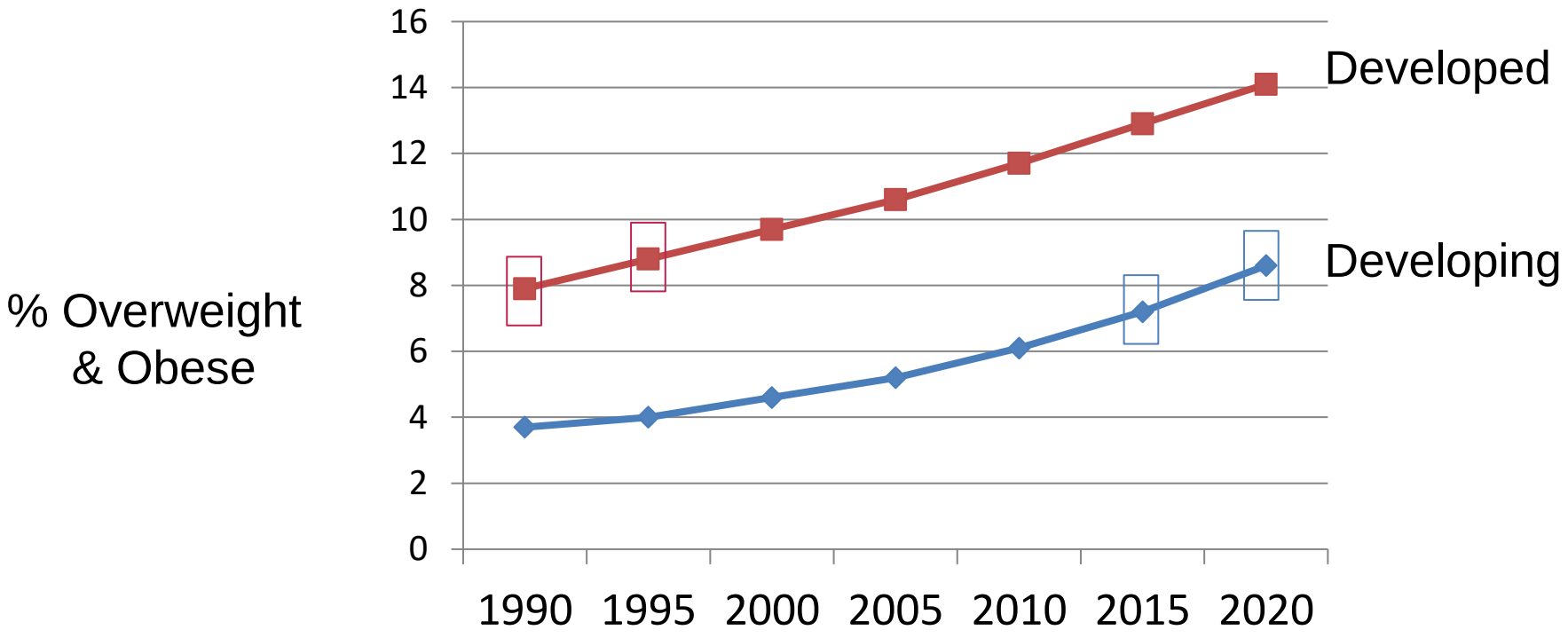
Source: *CDC Behavioral Risk Factor Surveillance System*

Obesity trends among U.S. children and adolescents



Sex- and age-specific BMI > 95th percentile, based on CDC growth charts

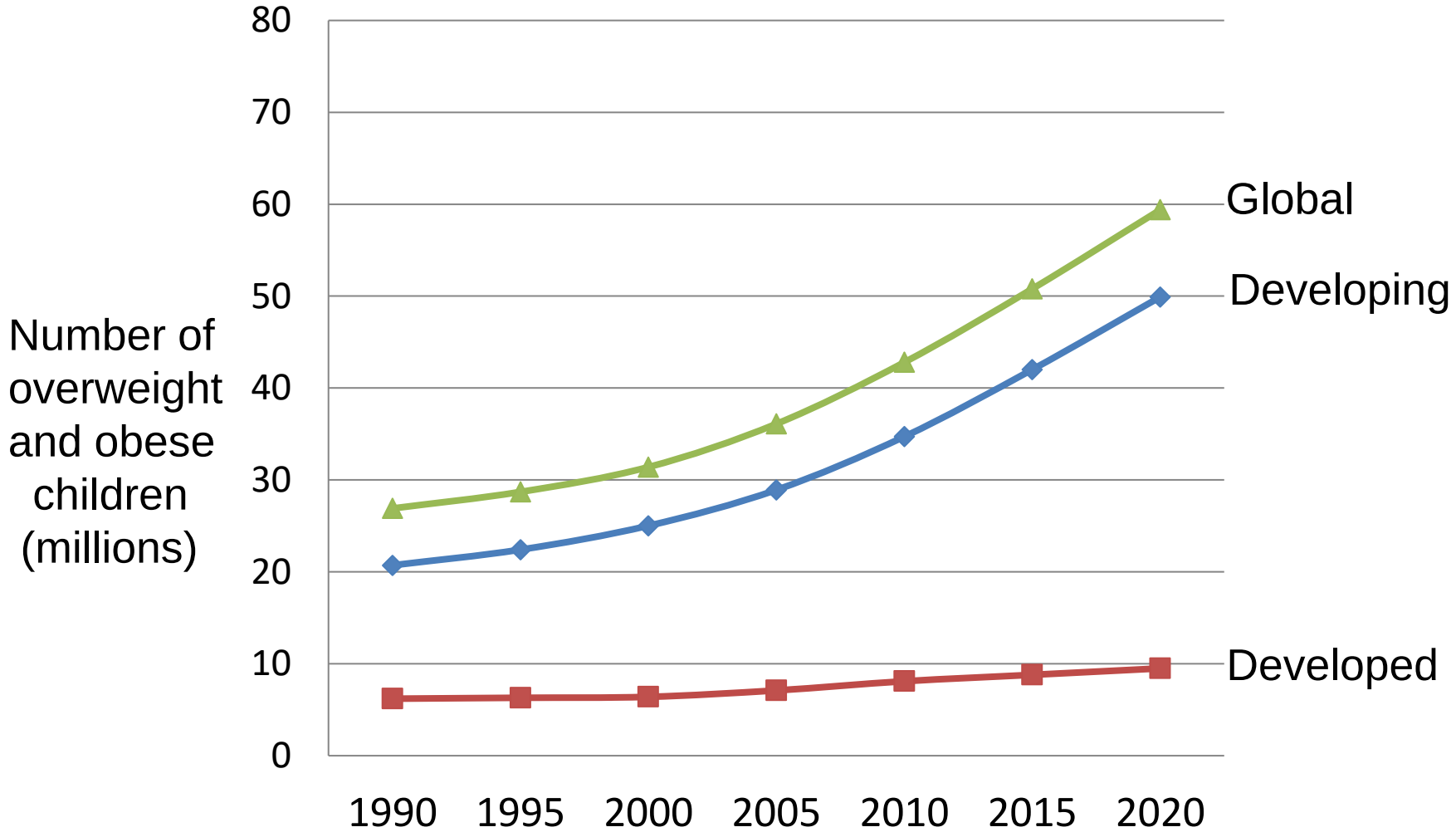
Comparison of time trends of overweight & obese children globally



Prevalence: (>2SD above weight-for-height median) in children 0-5 years old

M de Onis et al. *Am J Clin Nutrition* 2010; 92; 1257-64.

Global number of overweight & obese children <5, by locale



Number: (>2SD from weight-for-height median) in children 0-5 years old

M de Onis et al. *Am J Clin Nutrition* 2010; 92; 1257-64.

Is the obesity pandemic being driven
by a **perturbed early-life** microbiome?

Is the obesity pandemic being driven
by a **perturbed early-life** microbiome?

Is the obesity pandemic being driven
by an **antibiotic-perturbed early-life** microbiome?

Elements of the **metabolic** hypotheses

Humans and our microbiomes have evolved together.

The early life microbiota is a partner in normal host **metabolic** development.

Changes in the early life microbiome due to **antibiotic exposure**
affects the trajectory of **metabolic** development.

These changes are underlying **obesity** (varying in latency)
that has increased markedly in incidence in recent decades.

Correcting the microbiome deficits in an appropriate time window
can prevent the development of **obesity** in a proportion of individuals..

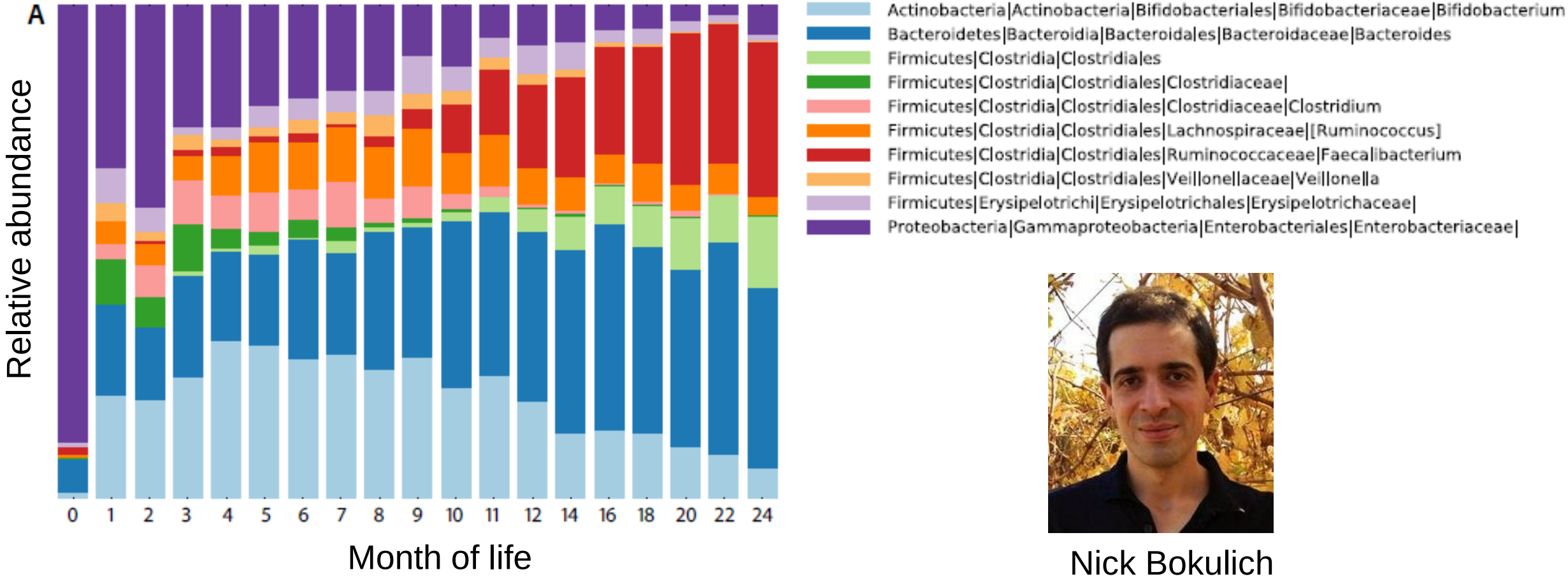
Bradford Hill criteria for establishing evidence of a causal relationship between a presumed cause and an observed effect.

Strength	effect size
Consistency	reproducibility
Specificity	the higher the specificity, the more likely a causal relationship
Temporality	the effect must occur after the cause
Biological gradient	dose-response relationship
Plausibility	a plausible mechanism is helpful
Coherence	concordance between epidemiological and laboratory findings
Experiment	for hypothesis testing
Analogy	similarities with other known associations

Reversibility	removing the cause should remove the effect

Sir Austin Bradford Hill, *Proc Royal Soc Medicine1965; 58: 295-300.*

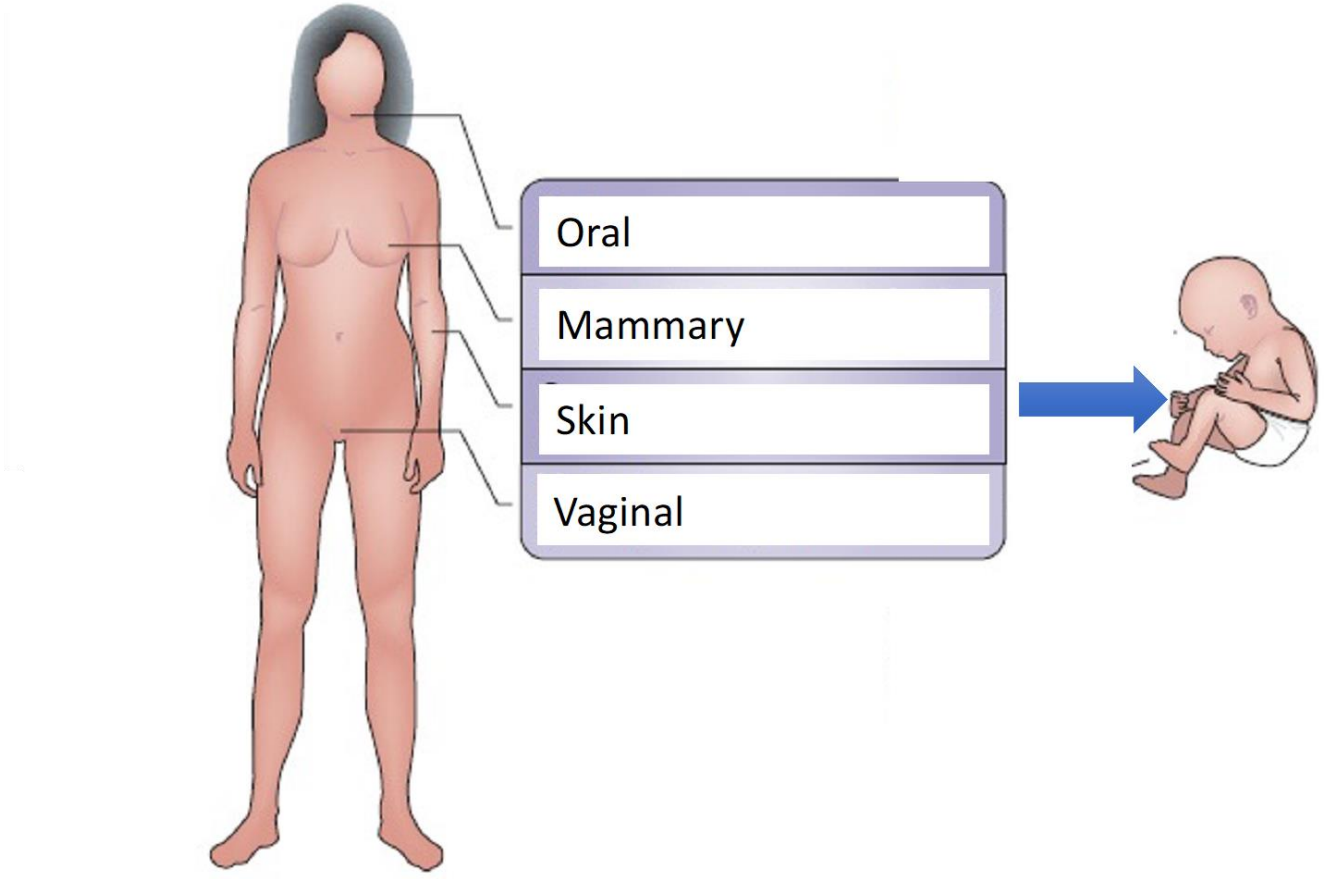
Predominant intestinal taxa in the first 2 years of life



Nick Bokulich

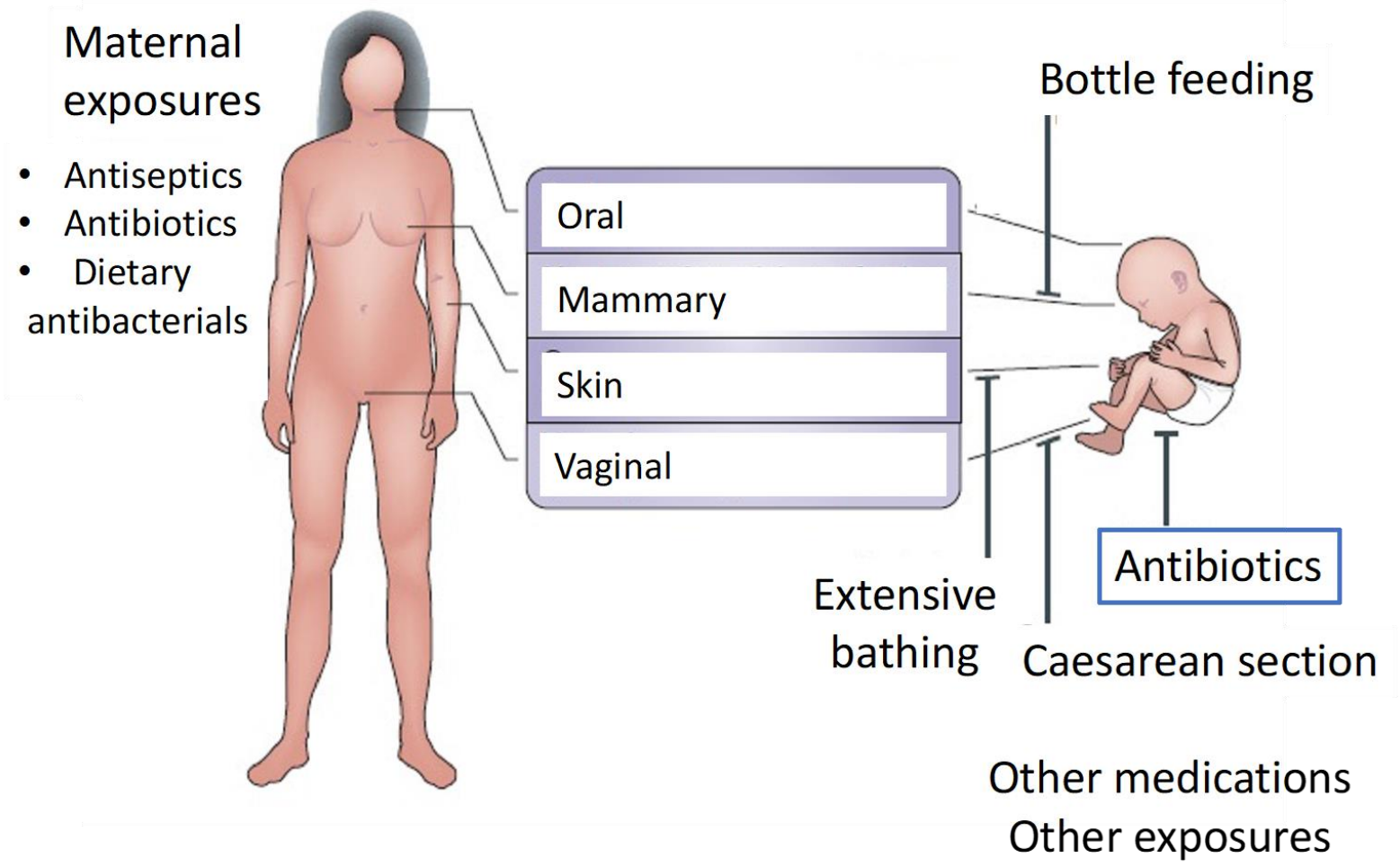
N. Bokulich *et al. Sci Trans Med* 2016; 8: 343

Mother→ Child Transfer of Microbes (Ancient)



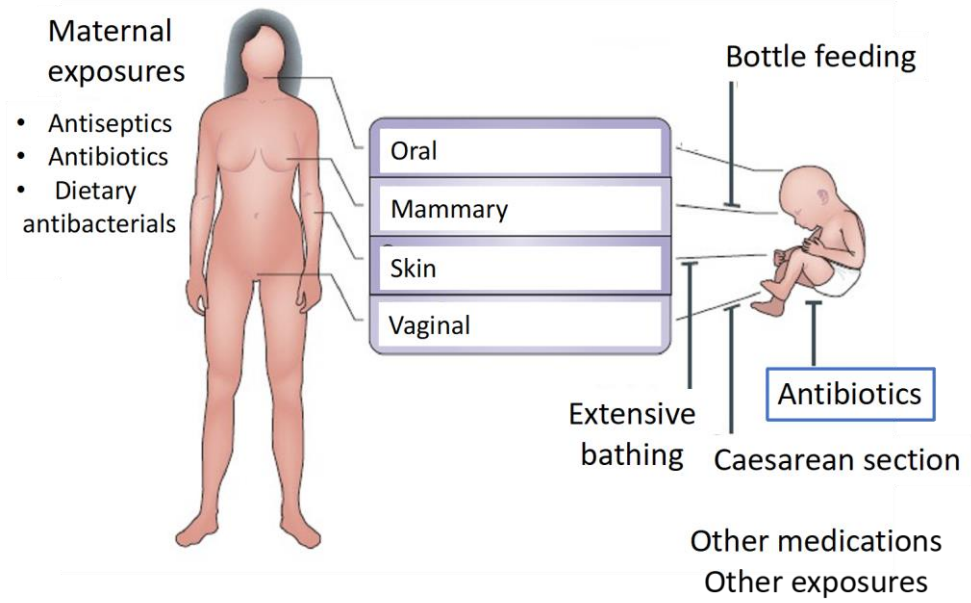
Nature Reviews Genetics 2012;13:260-70

Mother→ Child Transfer of Microbes (Modern)



MJ Blaser. *EMBO Reports 2006;* I Cho & MJ Blaser. *Nature Reviews Genetics* 2012

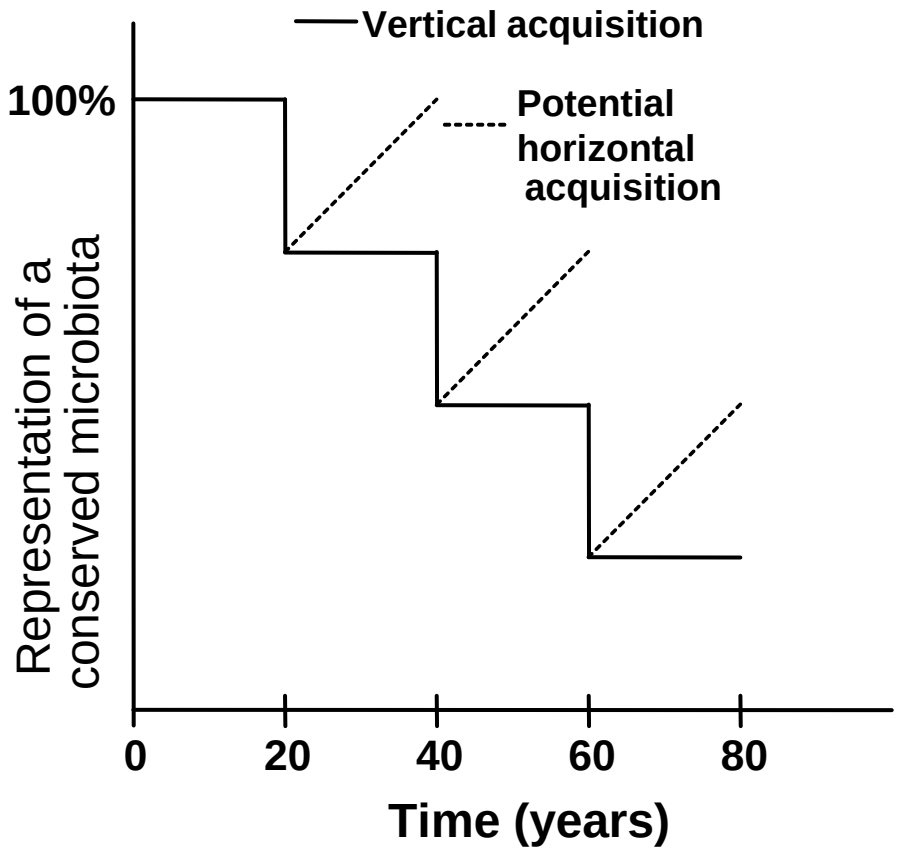
Theory of *Disappearing Microbiota*



- Changed human ecology has altered transmission and maintenance of ancestral microbes, which affects the composition of the microbiota.
- The microbes, both good and bad, usually acquired **early in life** are especially important, since they affect a developmentally critical stage.

Lancet 1997; *Gut* 1998; *Perspect Biol Med* 2002; *Scientific American* 2005; *EMBO Reports* 2006; *Nature Rev Microbiol* 2009; *Nature* 2011; Science 2016; *Nature Rev Immunol* 2017; *Cell* 2018

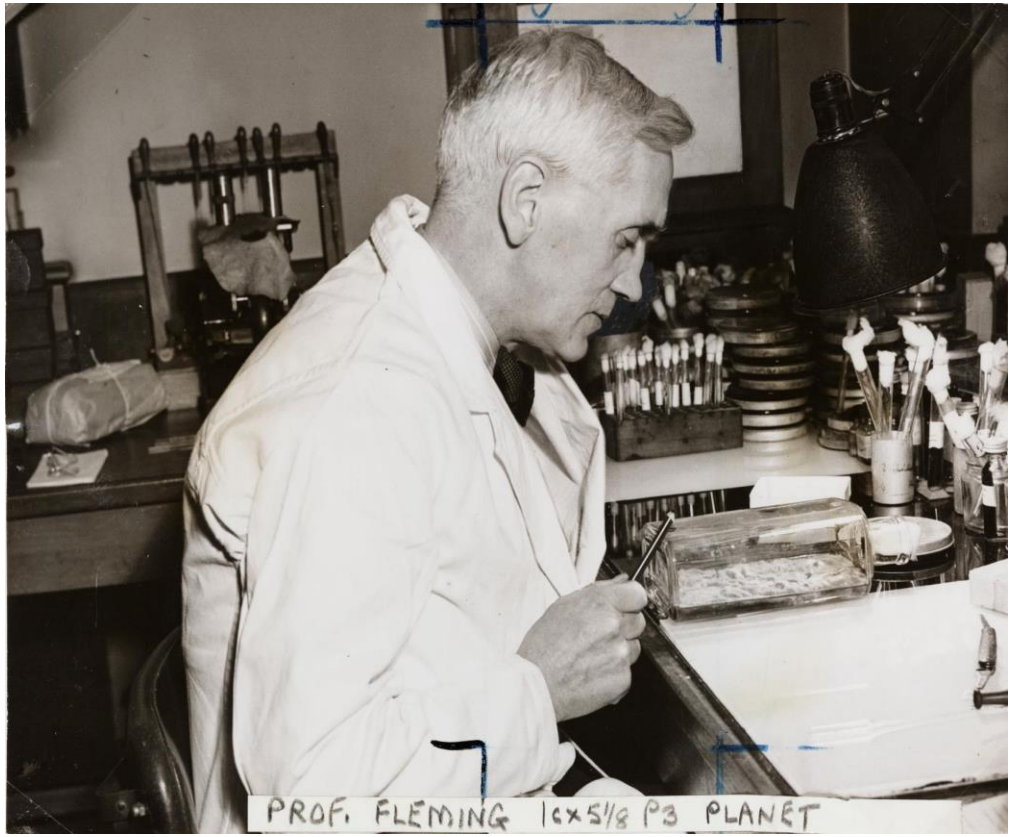
The effect of maternal status on the resident microbiota of the next generation



Loss of species and genes related to normal host development

Nature Rev Microbiol 2009;7:887

Antibiotics: among the greatest discoveries of the 20th century



Since the late 1940's → Saved innumerable lives + Revolutionized medicine

Scale of antibiotic exposures

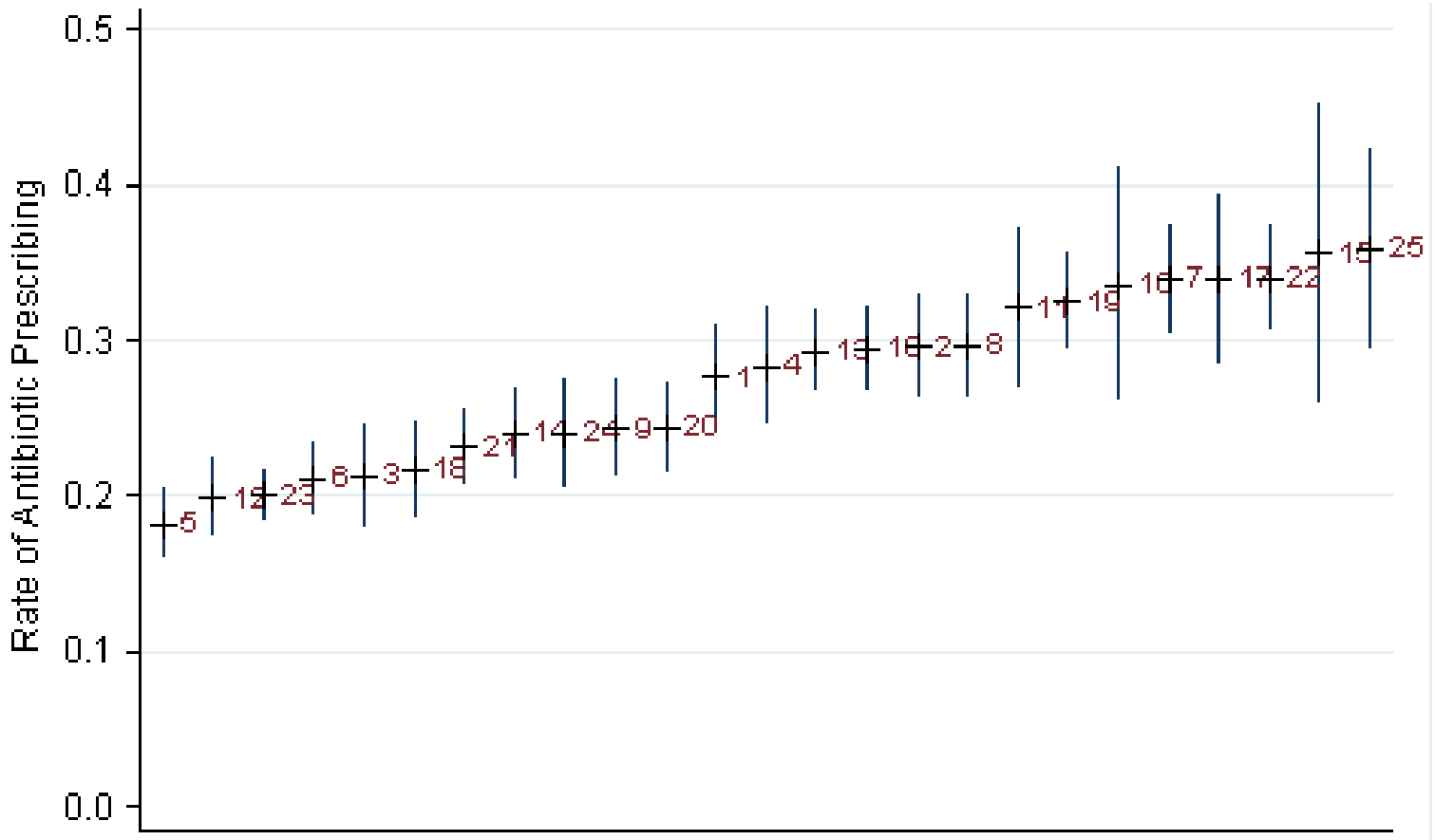
- >73 billion antibiotic doses worldwide yearly
- USA (2010): Children: 2.7 courses by 2 years; 11.0 by 10 years
  Pregnancy: >50% treated or given prophylaxis

+

Extensive variation in use

+ Exposures (unknown magnitude) from farm antibiotic use

Rates of antibiotic prescribing for sick children in 25 Pediatric practices



Excluding: preventive visits, CCC: Chronic Complex Conditions
Standardized by: age, sex, age-sex, race, Medicaid status

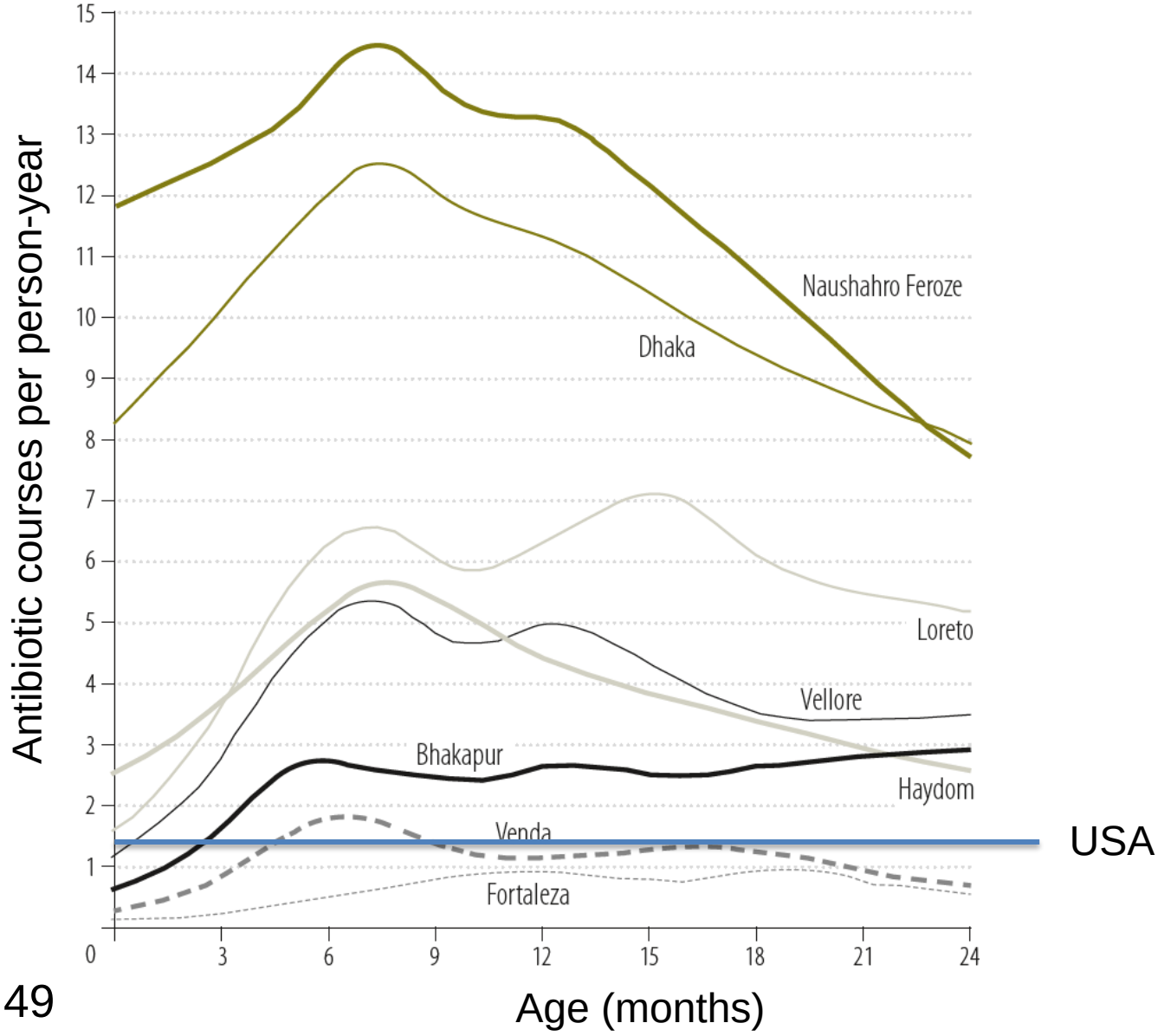
JS Gerber et al. *J Pediatric Infect Dis* 2015

Antibiotic use in the first 2 years of life among children in the MAL-ED birth cohorts

Number of children: 2134

Number of study sites: 8

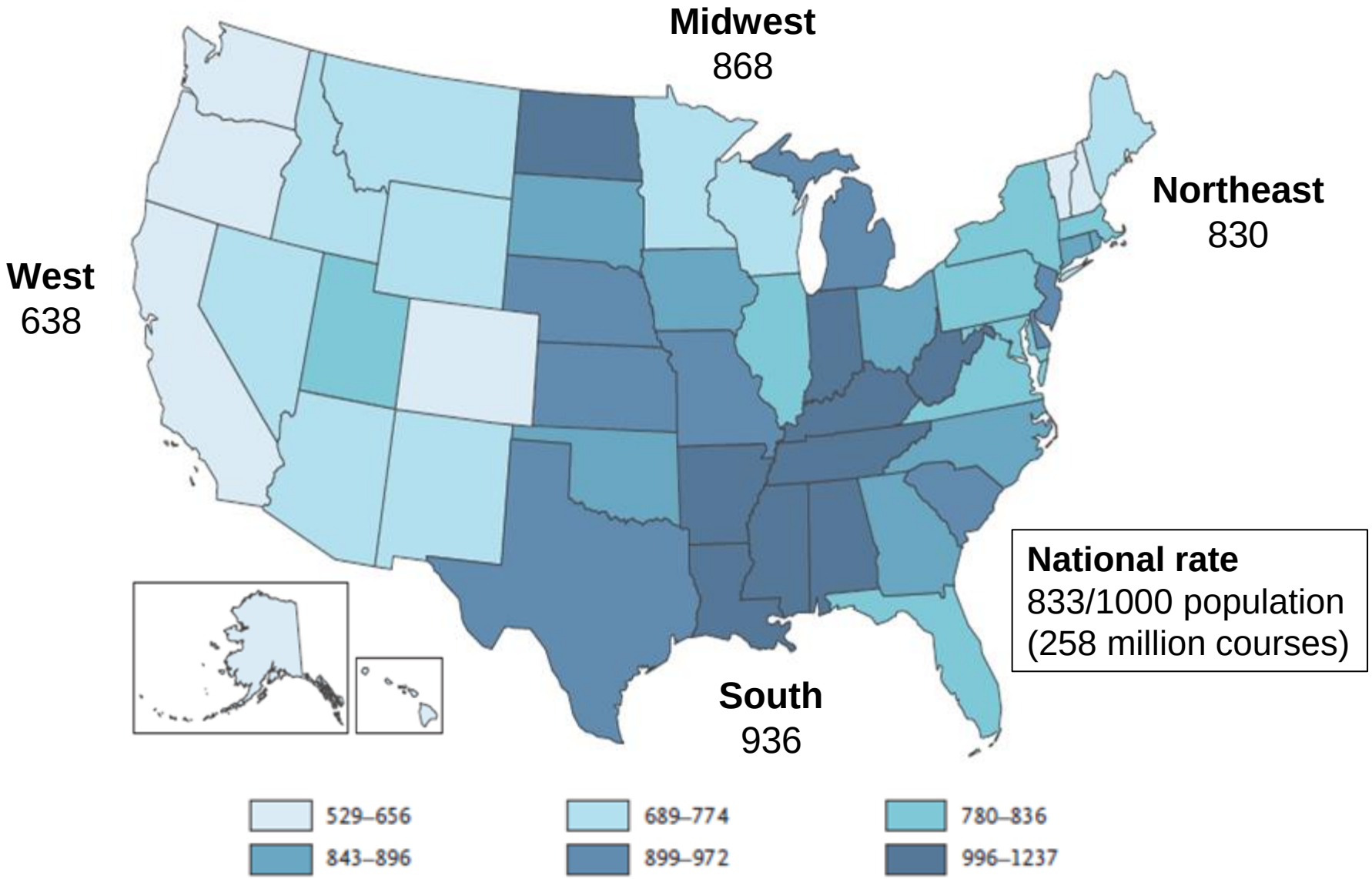
Years: 2009-14



ET Rogawski *et al. Bull WHO* 2017; 95: 49

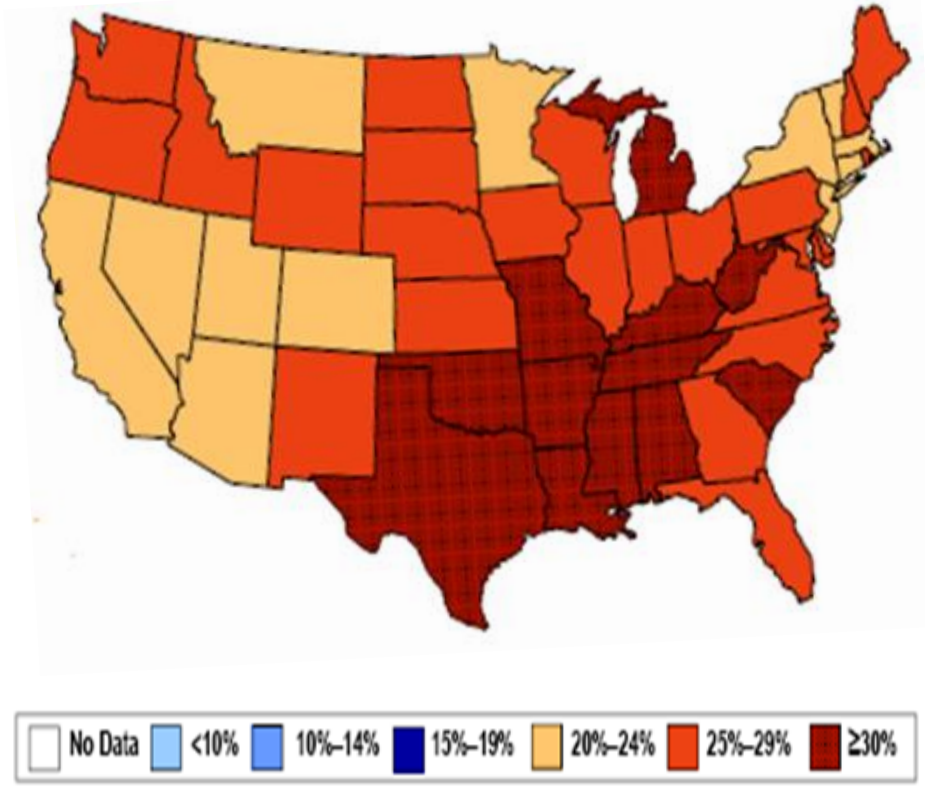
1. Is early life antibiotic exposure associated with subsequent disease risk in populations?

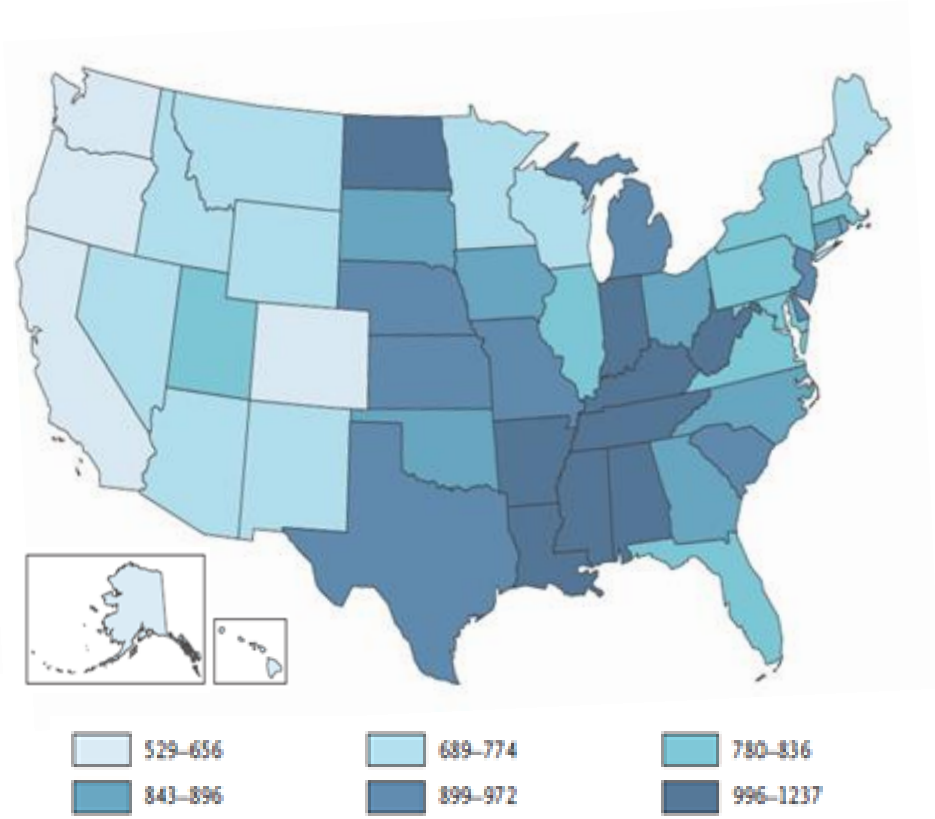
Outpatient antibiotic usage rates by region, 2010



L Hicks et al. *N Engl J Med* 2013, 368:1461.

Ecologic association: comparisons between the geography of obesity and antibiotic use in populations, 2010





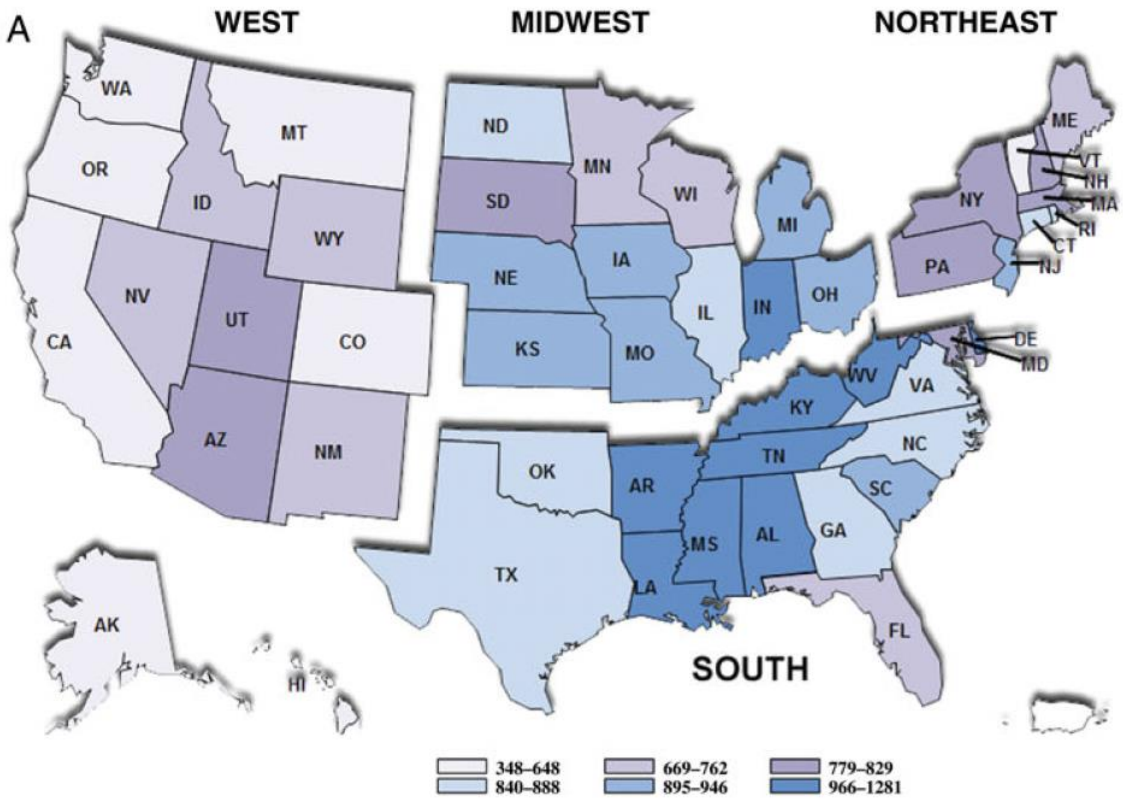
Antibiotic prescriptions per 1000 persons, 2010

L Segal & MJ Blaser, *Ann Am Thor Soc* 2014

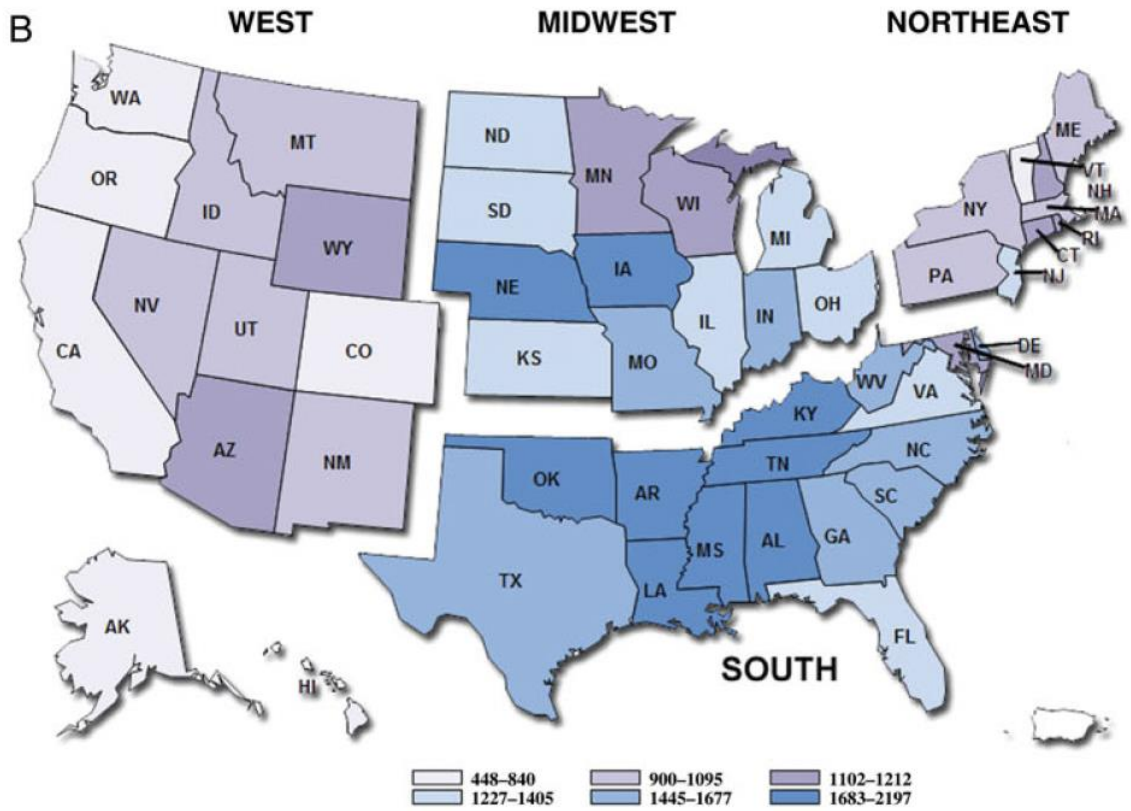
Observational data

Antibiotic prescribing in 2011 per capita by state and by age group

All ages



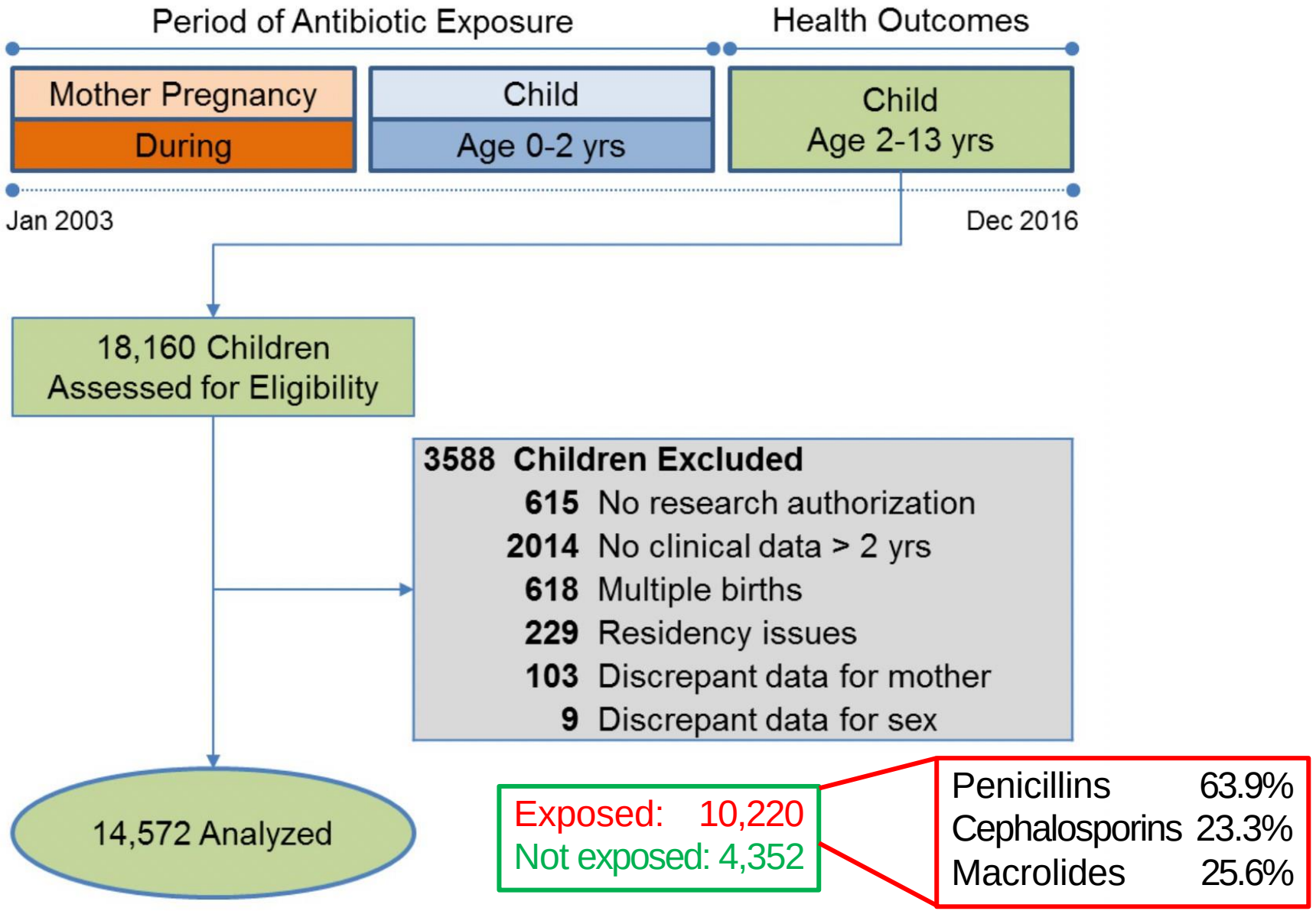
Age ≤2 years



L Hicks *et al. Clin Infect Dis* 2015

2. Is early life antibiotic exposure associated with subsequent disease risk in individuals?

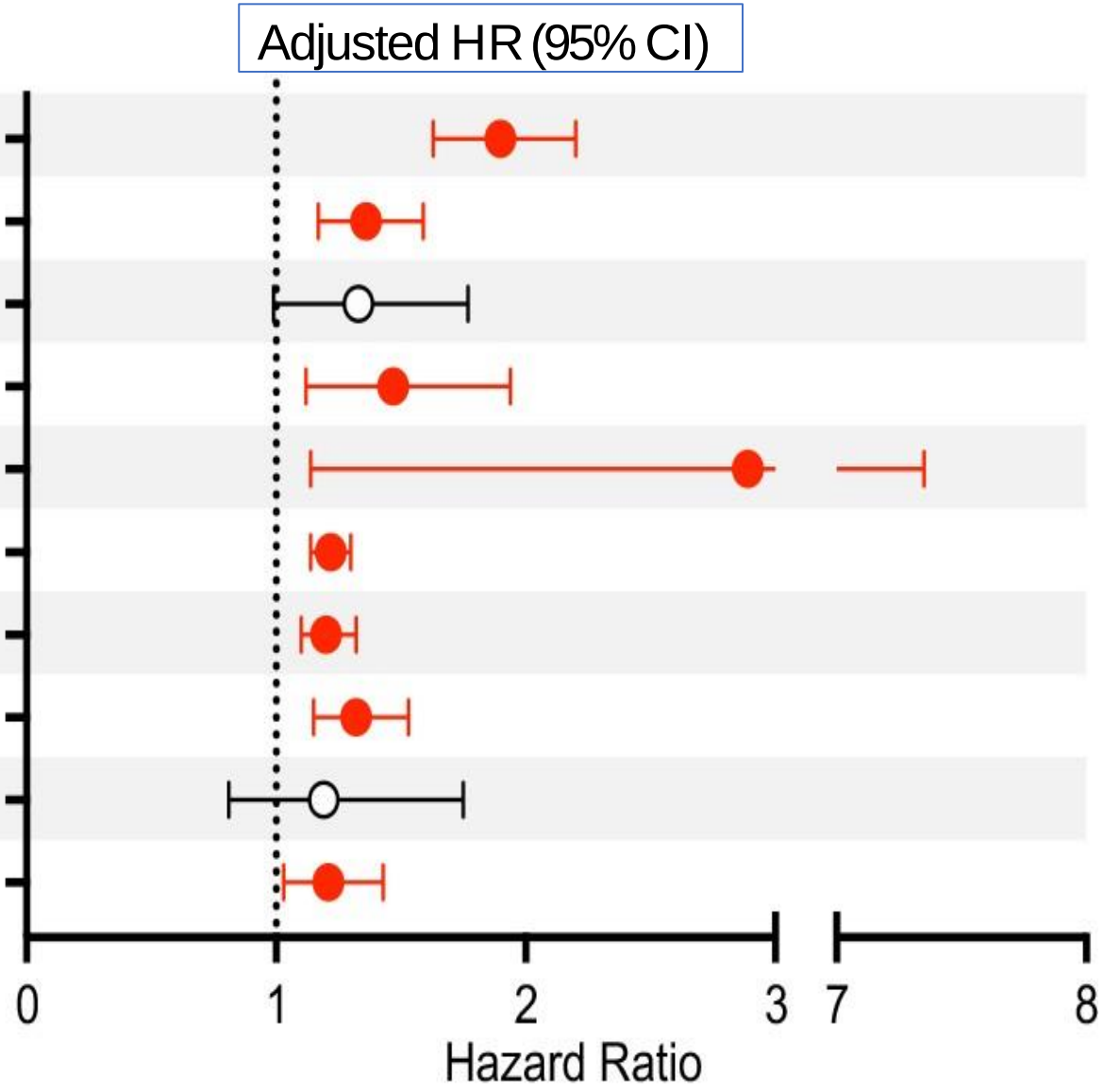
Mayo Clinic/Olmsted County study to assess associations of early life antibiotic exposure with subsequent childhood health conditions, 2003-2016



Z Aversa *et al. Mayo Clin Proc* 2021; 96:66-77

Antibiotic exposure before age 2 and risk of 10 common health conditions with childhood onset

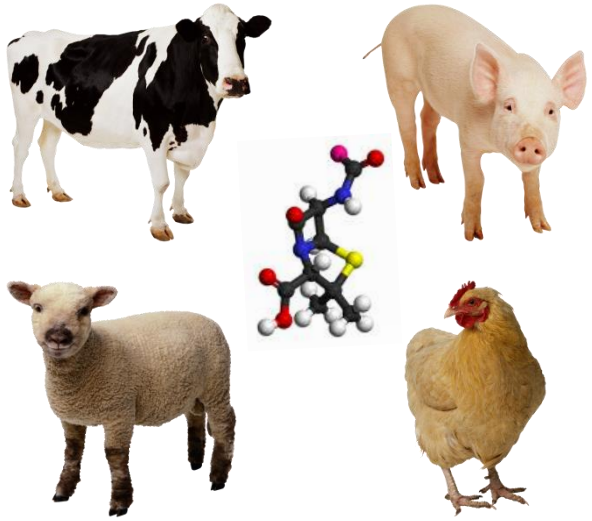
Condition	No. of subjects	No. of events	HR (95% CI)	p-value
Asthma	14299	1176	1.90 (1.63-2.20)	<.001
Allergic rhinitis	14545	971	1.36 (1.17-1.59)	<.001
Food allergy	14402	261	1.33 (0.99-1.77)	.054
Atopic dermatitis	14166	297	1.47 (1.12-1.94)	.006
Celiac disease	14569	45	2.89 (1.14-7.35)	.025
Overweight	12873	4856	1.22 (1.14-1.30)	<.001
Obesity	13649	2567	1.20 (1.10-1.32)	<.001
ADHD	14572	1085	1.32 (1.15-1.53)	.001
Autism	14571	142	1.19 (0.81-1.75)	.385
Learning disability	14392	751	1.21 (1.03-1.43)	.022



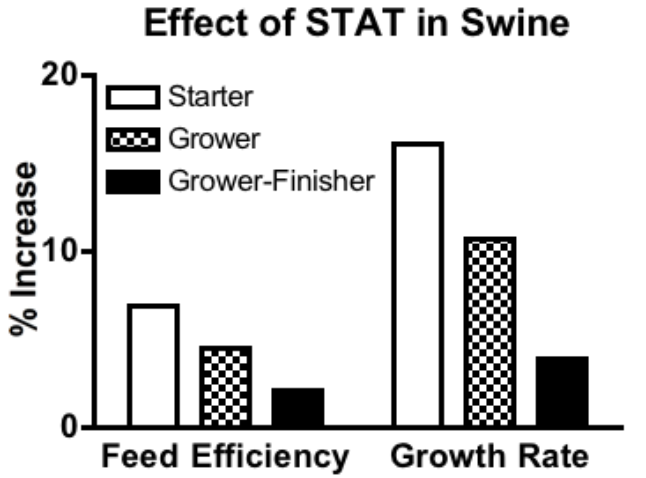
Specific associations with number of antibiotic courses, timing of exposure, and antibiotic class

3. Do antibiotic-induced perturbations cause the clinical conditions?

Antibiotics used in farm animals to promote their growth



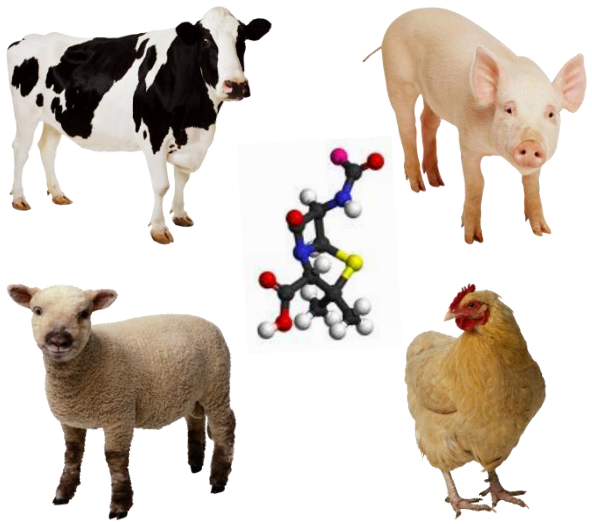
Antibiotic	Class	Target
Bambermycin	Glycolipid	Cell wall
Virginiamycin	Streptogrammin	Protein synthesis
Avilamycin	Orthosomycin	Protein synthesis
Bacitracin	Cyclic peptide	Cell wall synthesis
Monensin	Ionophore	Cell membrane
Carbadox	Quinoxaline	DNA Synthesis



(Adapted from Zimmerman, J Animal Sci, 1986)

Analogy

Experimentation: using mice to examine the effects of antibiotics



Antibiotic	Class	Target
Bambermycin	Glycolipid	Cell wall
Virginiamycin	Streptogrammin	Protein synthesis
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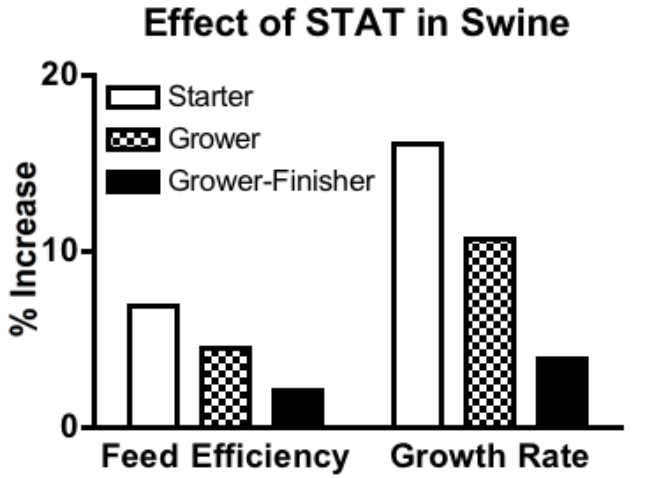
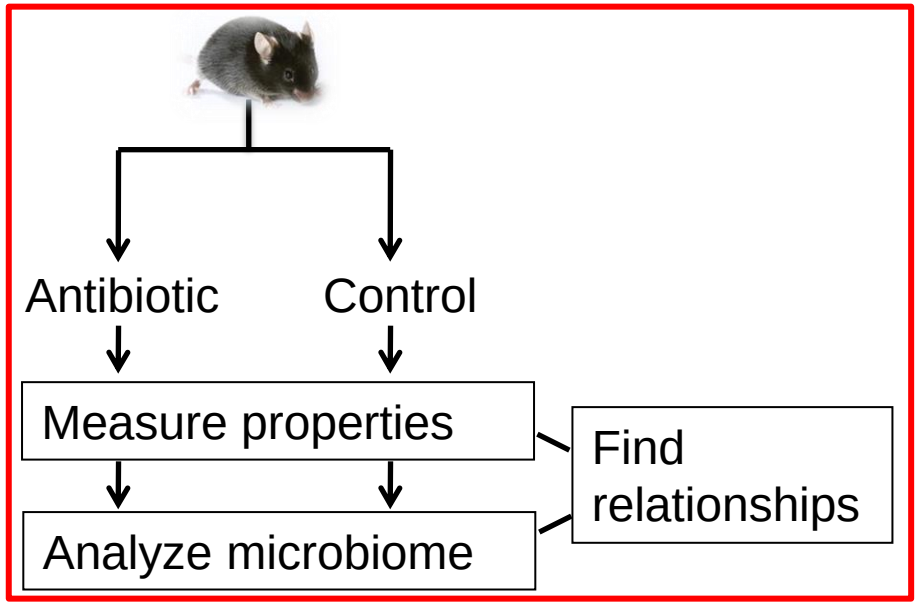
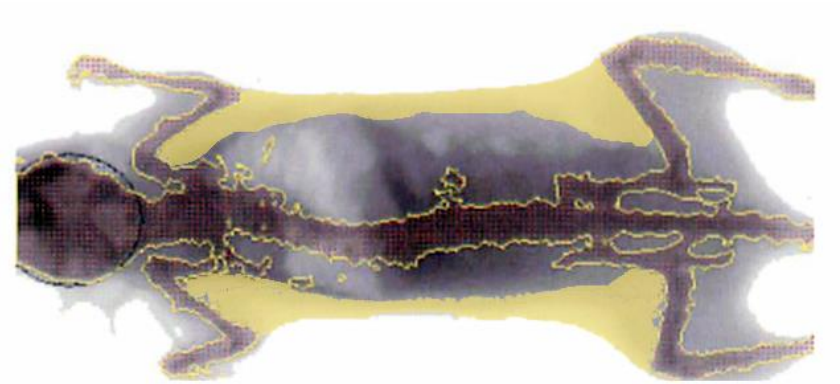
Bacitracin	Cyclic peptide	Cell wall synthesis
Monensin	Ionophore	Cell membrane
Carbadox	Quinoxaline	DNA Synthesis



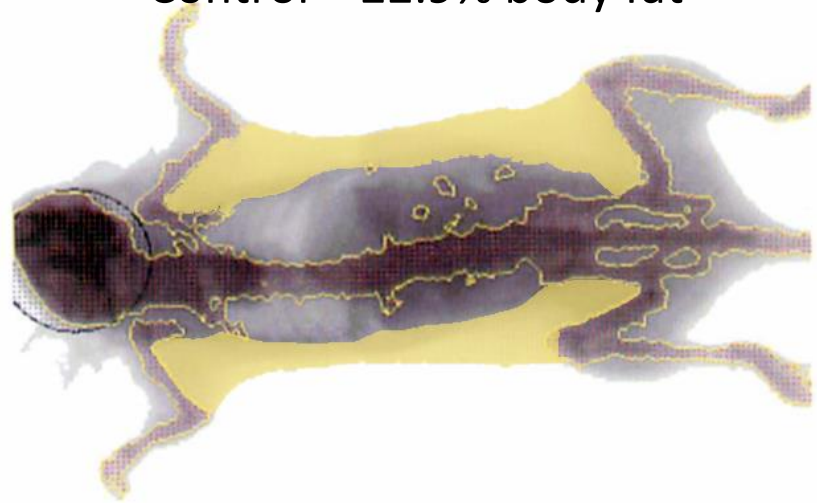
(Adapted from Zimmerman, J Animal Sci, 1986)



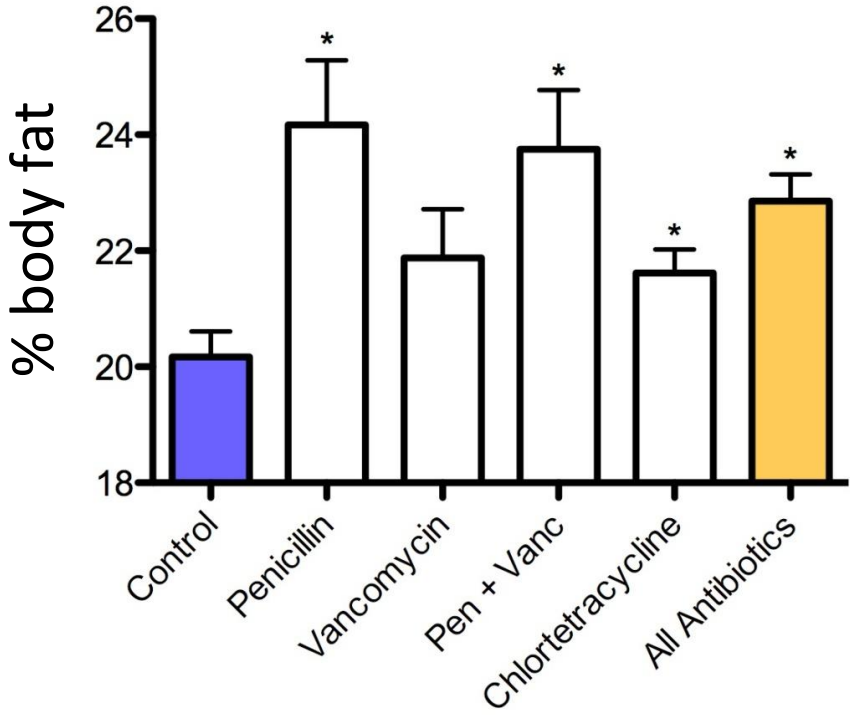
Body fat in antibiotic-exposed and control 10-week old mice



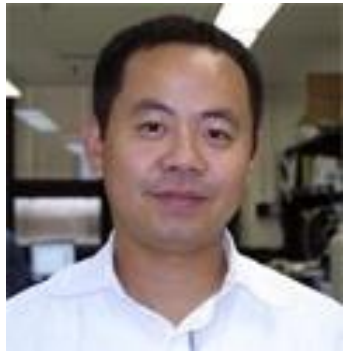
Control – 22.9% body fat



Antibiotic – 32.0% body fat



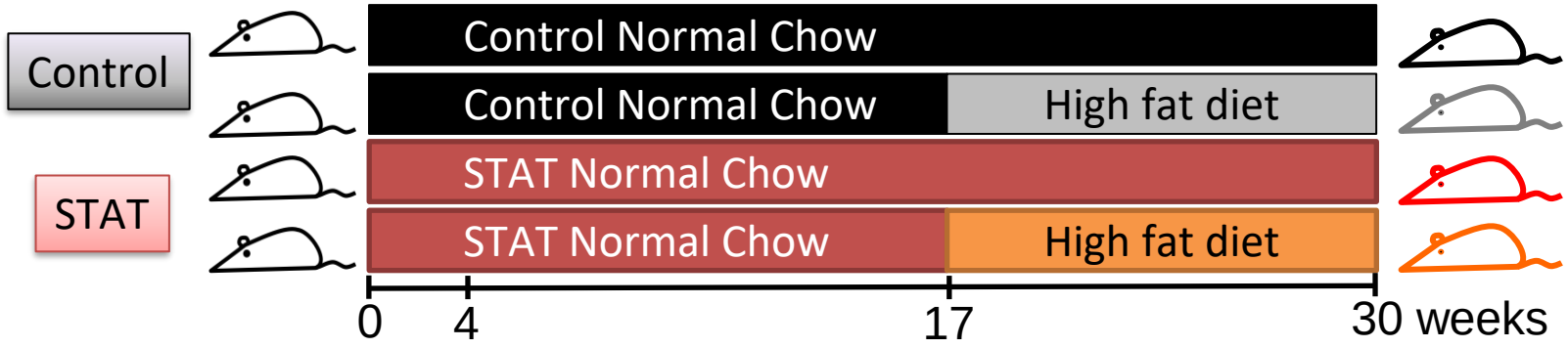
*p<0.05



Ilseung Cho

I Cho *et al. Nature* 2012; 488:621-6

Effects of combining high fat diet and antibiotics



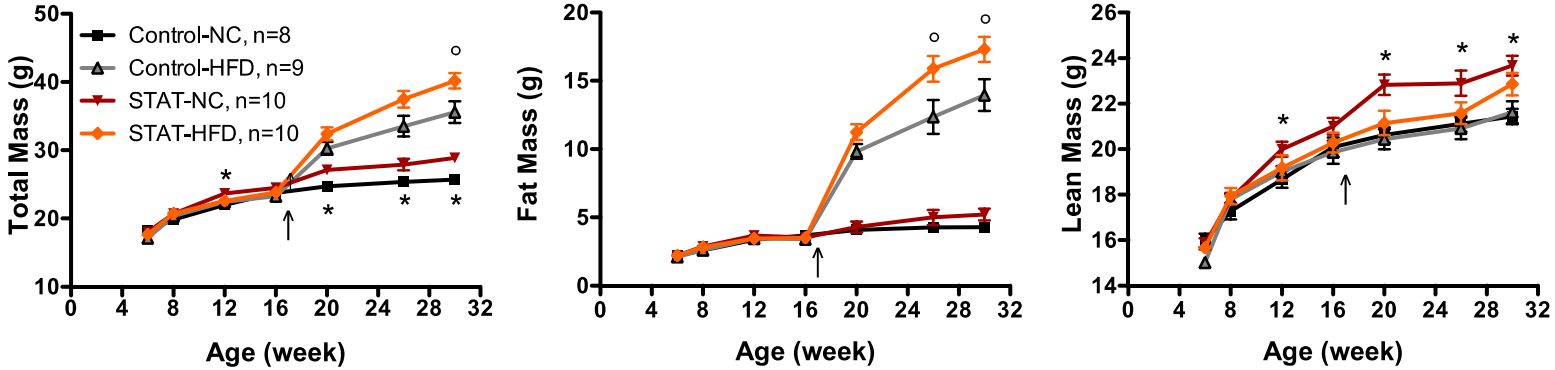
STAT = low-dose antibiotic exposure, as used on the farm

Laurie Cox

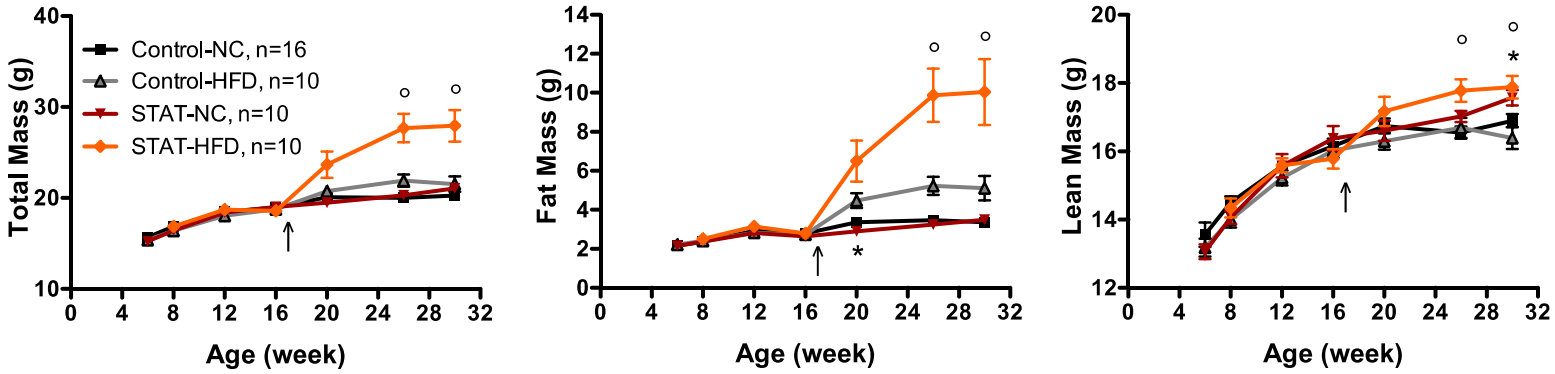
Cell 2014;158:705-21

HFD and antibiotic both contribute to body fat

Male

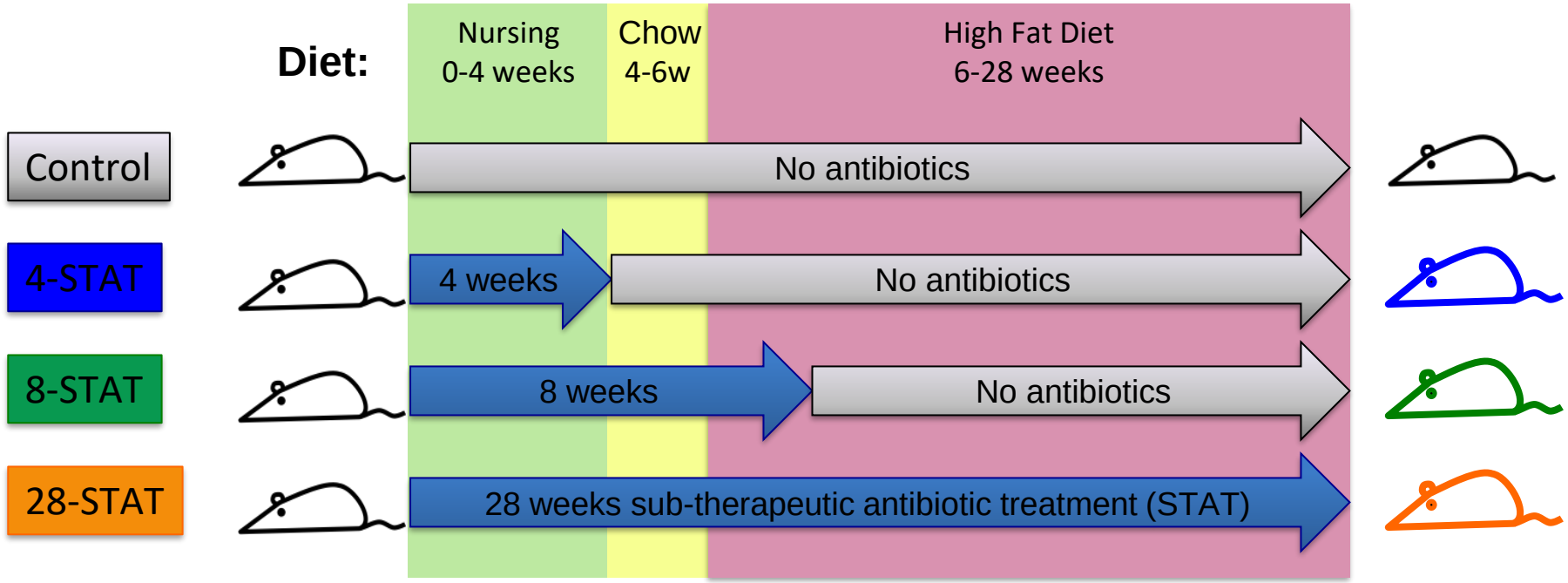


Female



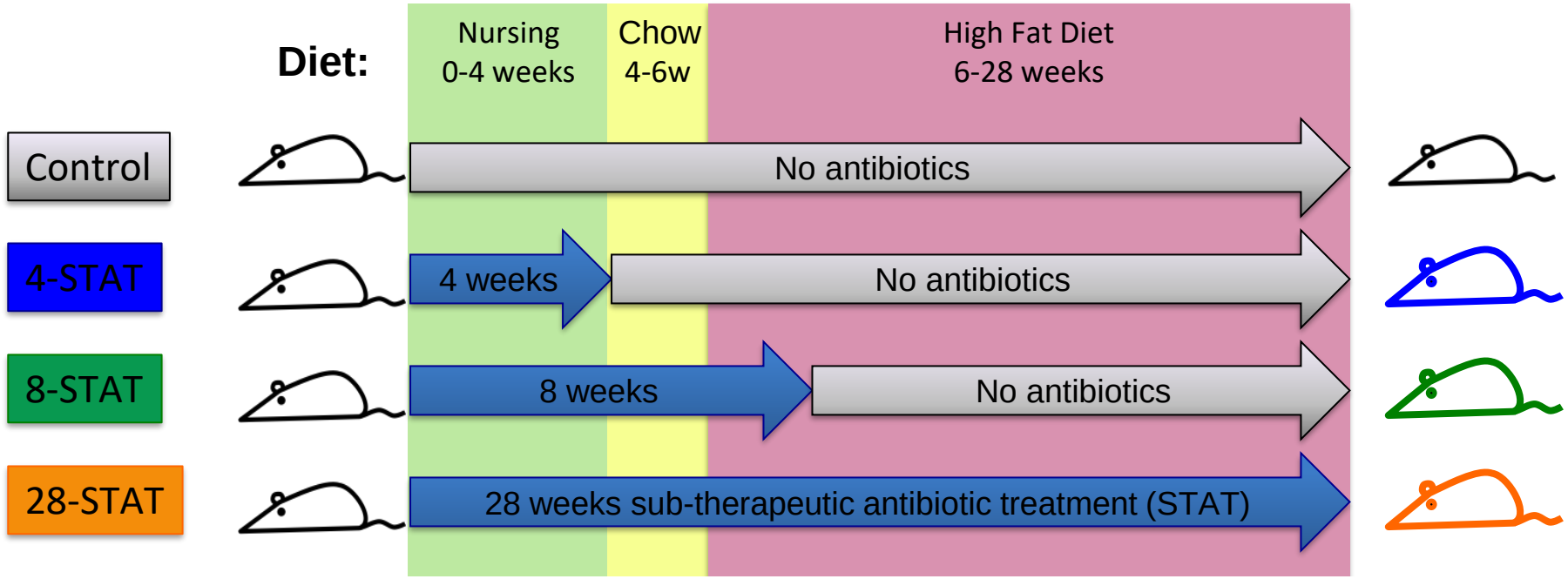
↑ High fat diet introduced * $p < 0.05$ NC ° $p < 0.05$ HFD

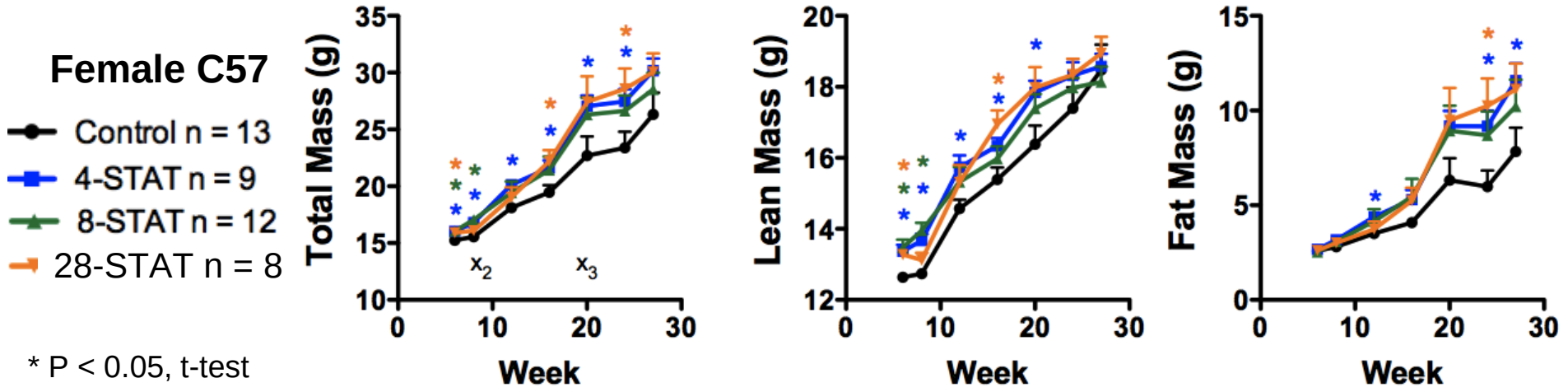
DuraSTAT: Are the metabolic changes **durable** with limited antibiotic exposure?



LN Cox *et al. Cell* 2014;158:705-21

Morphometric changes with limited antibiotic exposure





* P < 0.05, t-test

X_2: sacrificed 4 control and 4 8-STAT

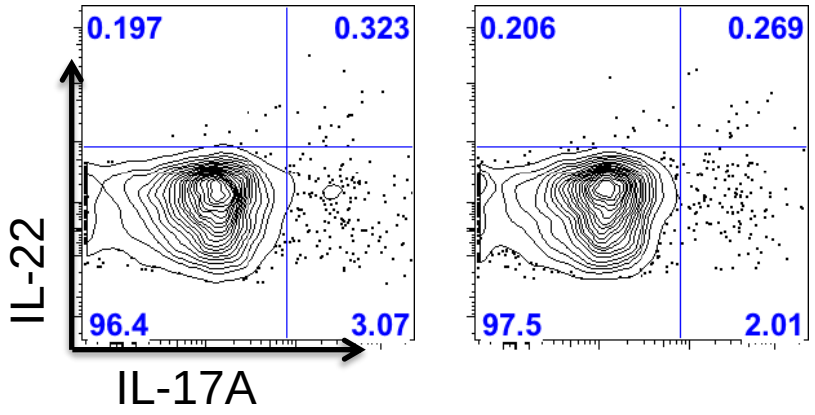
X_3: sacrificed 3 control and 3 28-STAT

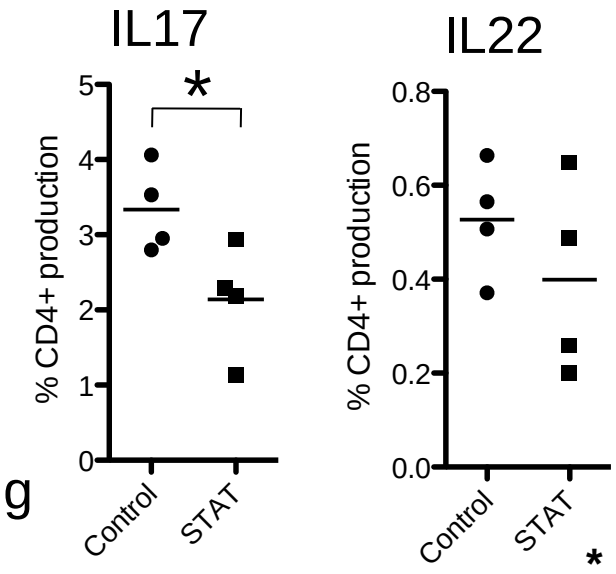
Analogy with the farm

Effects of STAT on intestinal Th17 populations

Small intestine

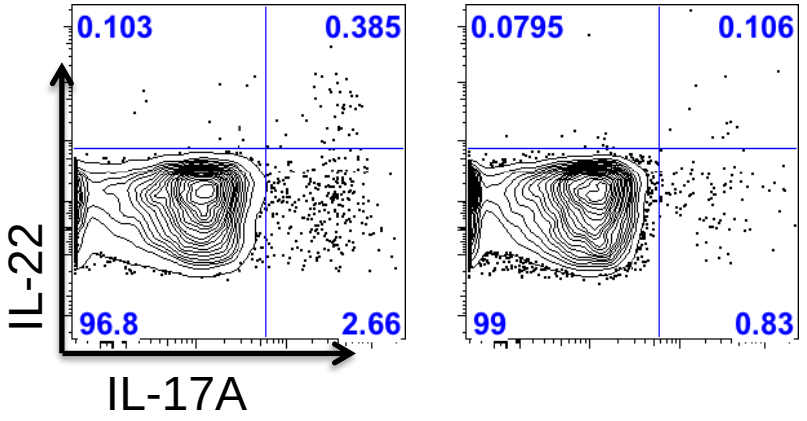
Control STAT

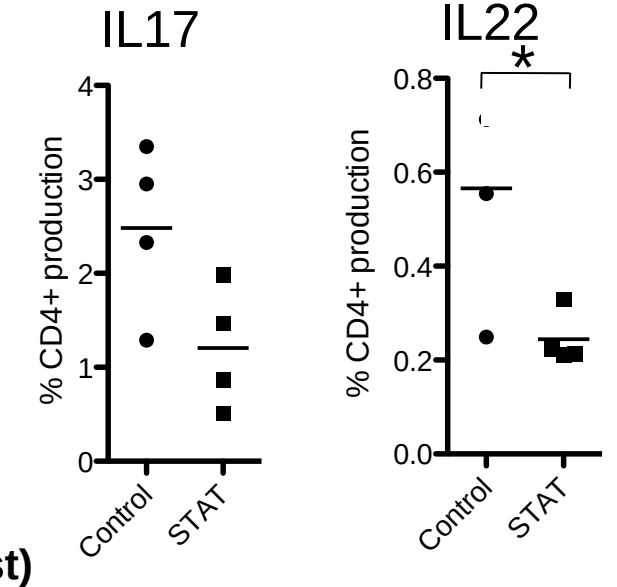




Large intestine

Control STAT

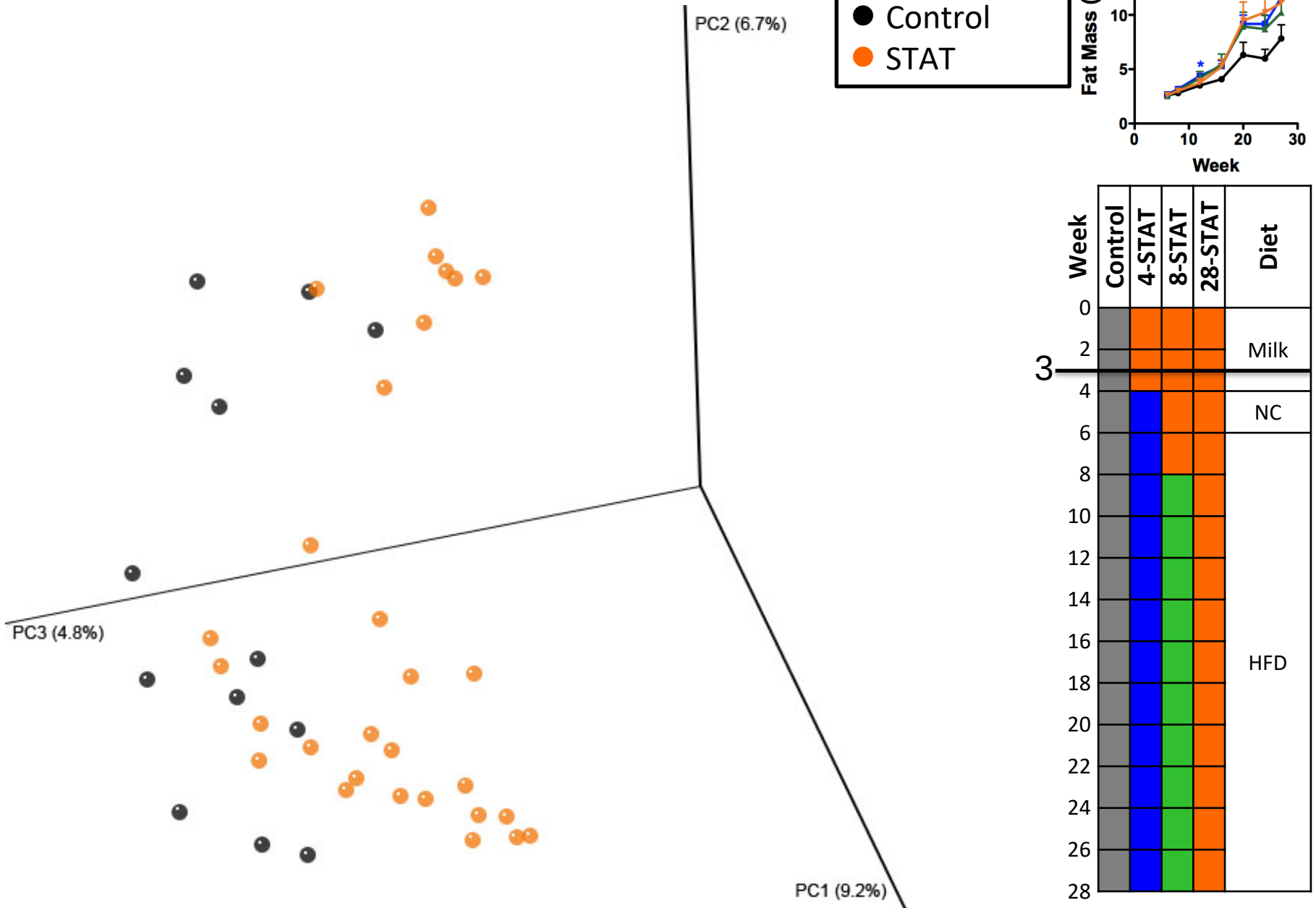




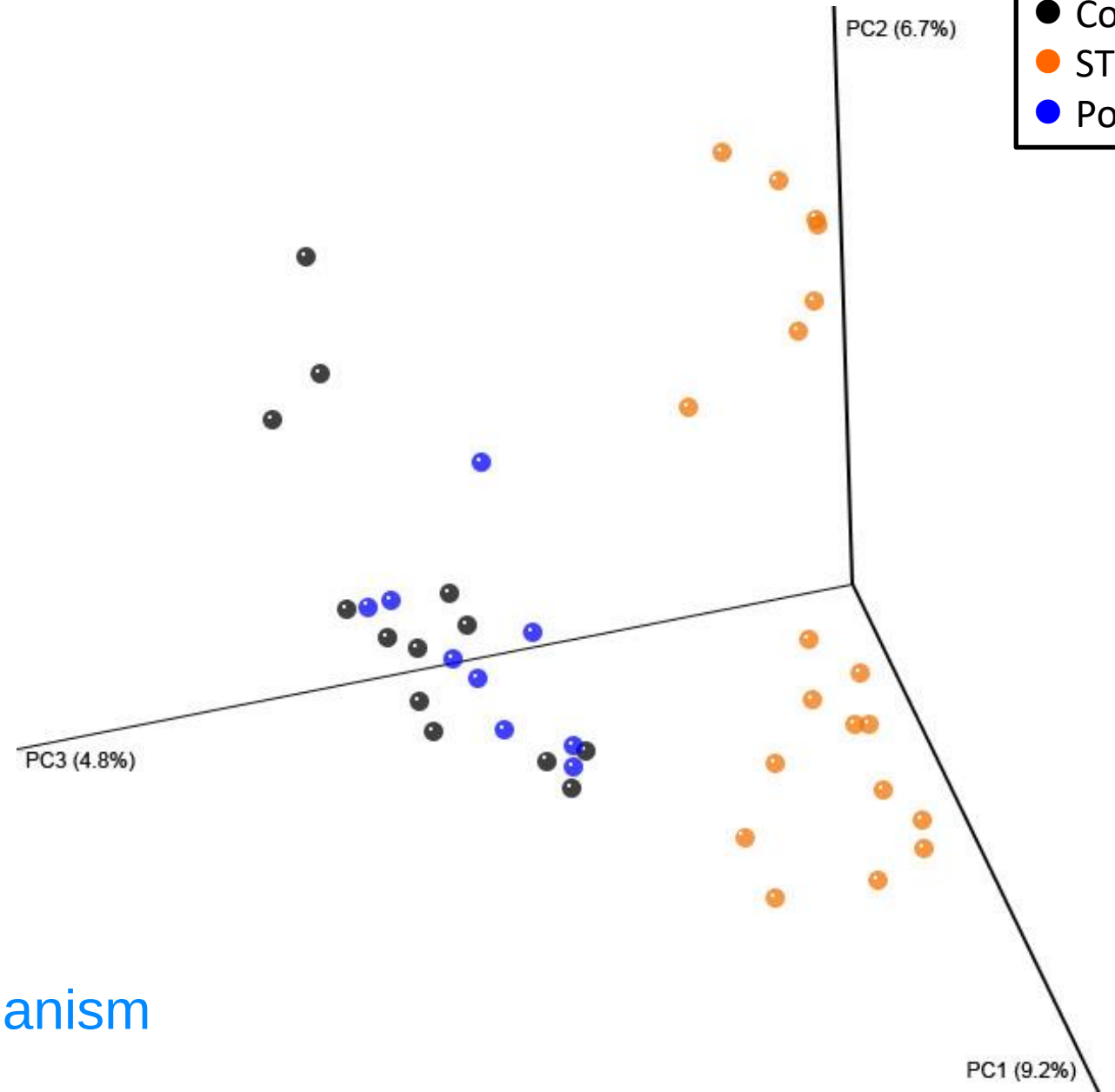
Jacqueline Leung
P'ng Loke Lab

*** p<0.05 (t-test)**

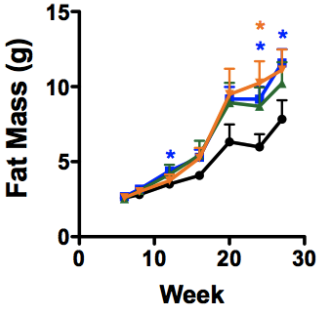
Fecal community structure at 3 weeks

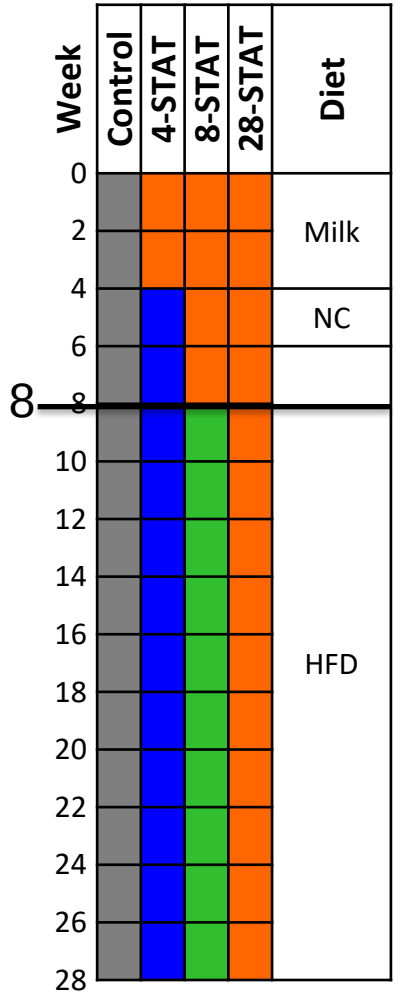


Fecal community structure at 8 weeks



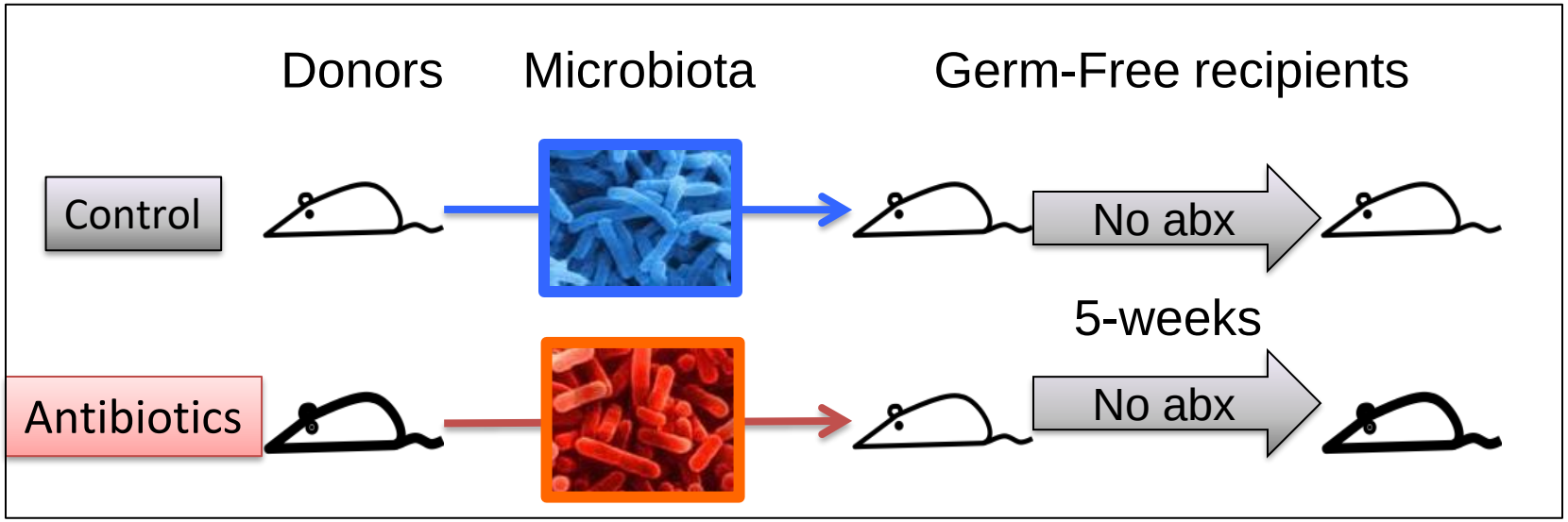
Control
STAT
Post 4-STAT

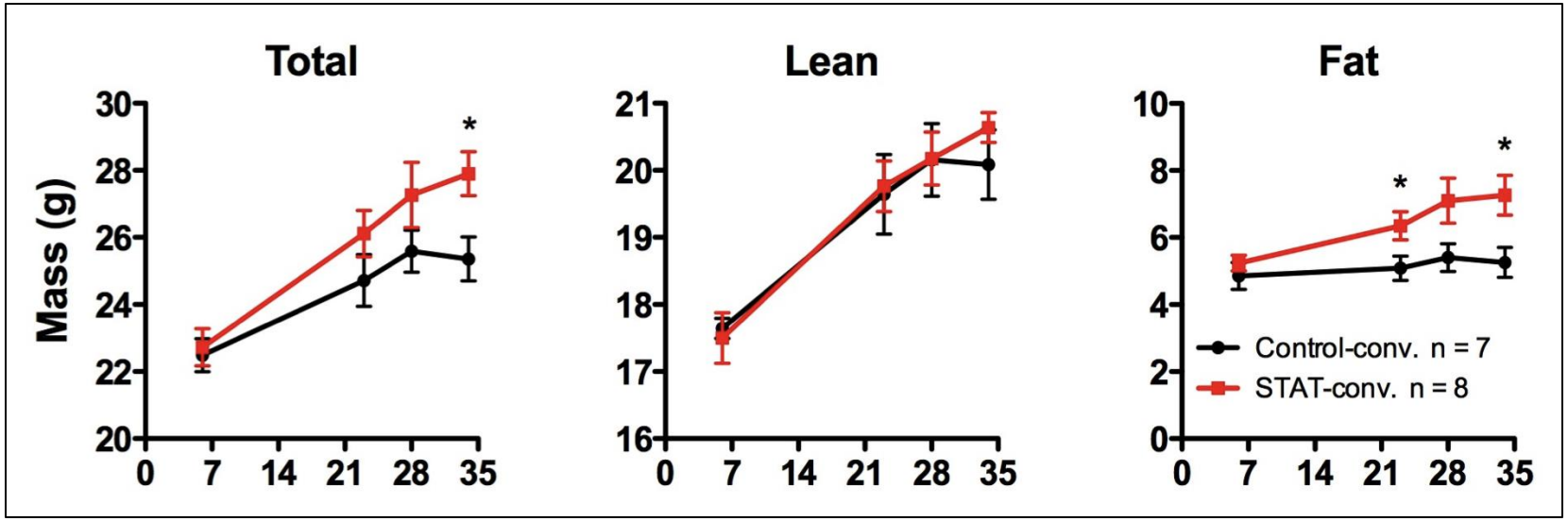




Mechanism

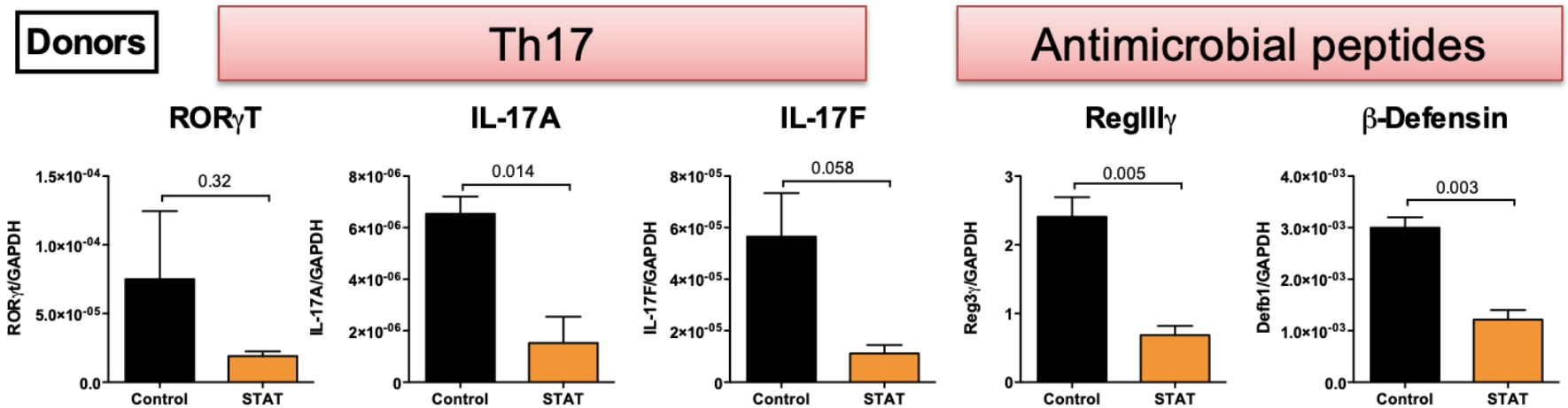
Experiment: Is microbe-induced obesity transferable?





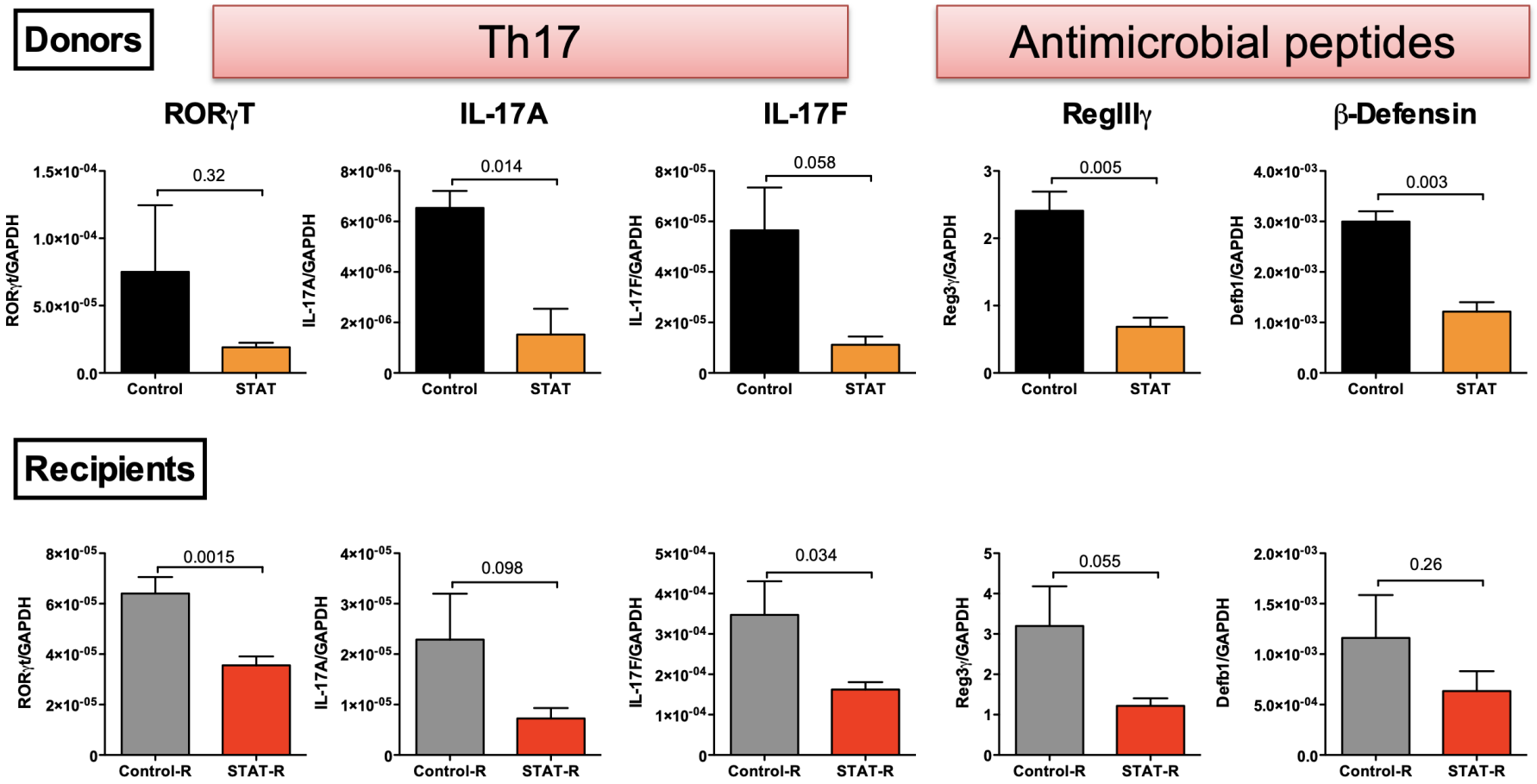
Body composition - Days post-transfer

Expression of genes involved in intestinal defenses in the microbiota of donor mice



p-values, by t-test

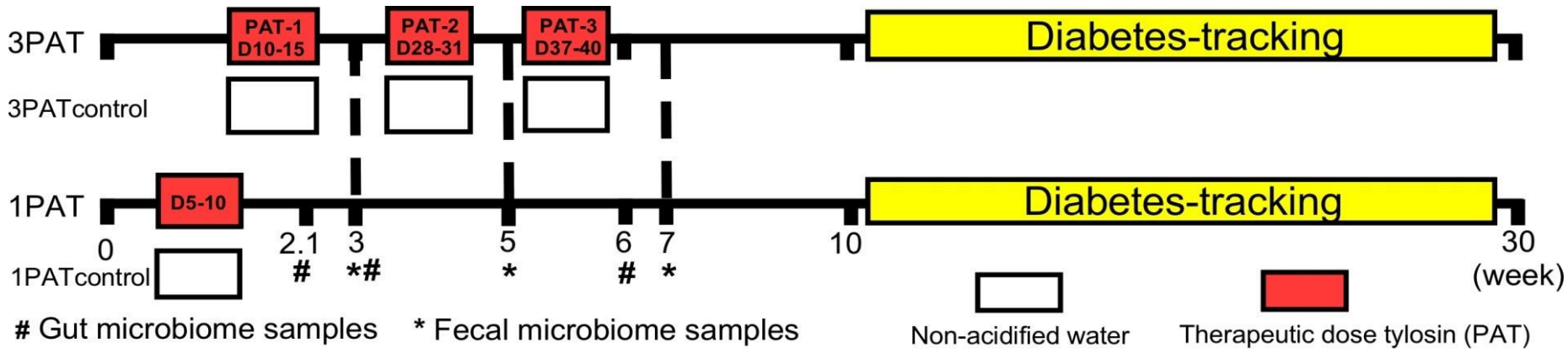
Expression of genes involved in intestinal defenses in the microbiota of donor and recipient mice



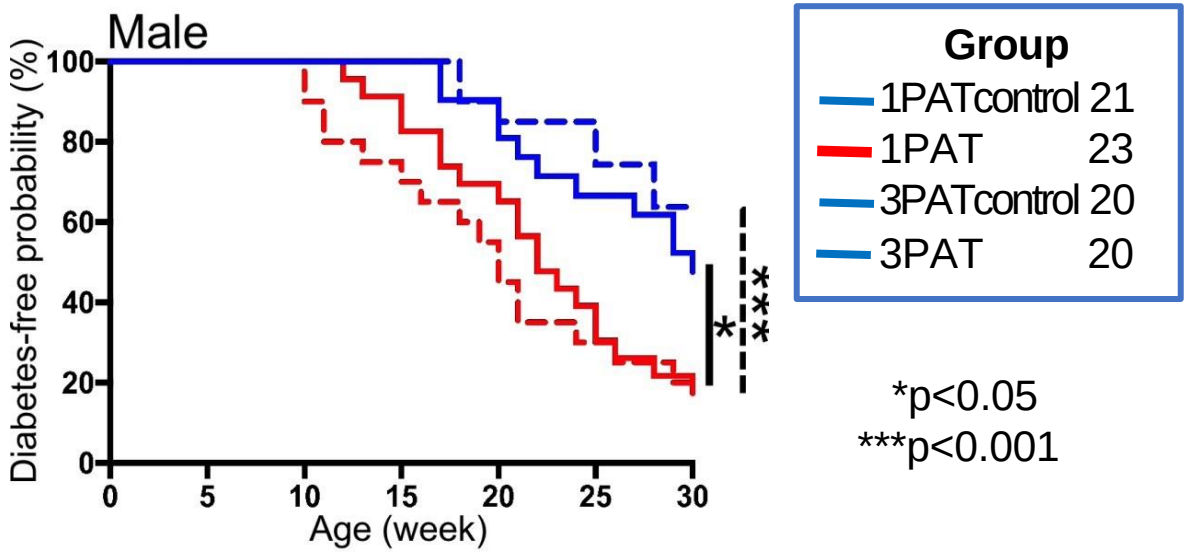
Mechanism

p-values, by t-test

Effect of antibiotic courses on Type 1 Diabetes development in NOD mice



Kaplan-Meier analysis of T1D incidence



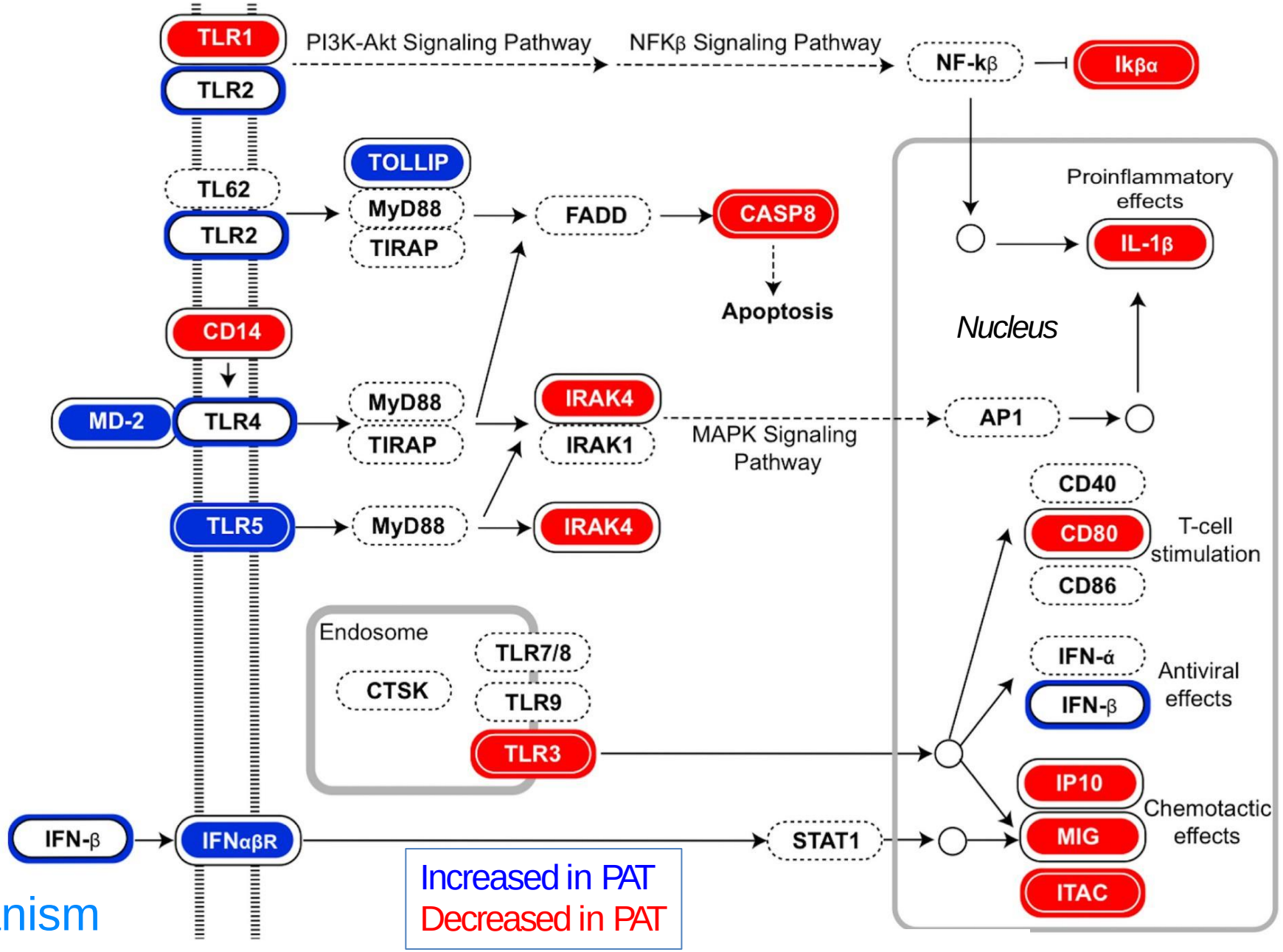
Group	
1PATcontrol	21
1PAT	23
3PATcontrol	20
3PAT	20

*p<0.05
***p<0.001

Xue-Song Zhang *et al.*

eLife: 2018

Mechanism: Differential TLR signaling pathway expression in the ileal wall (P12-23)

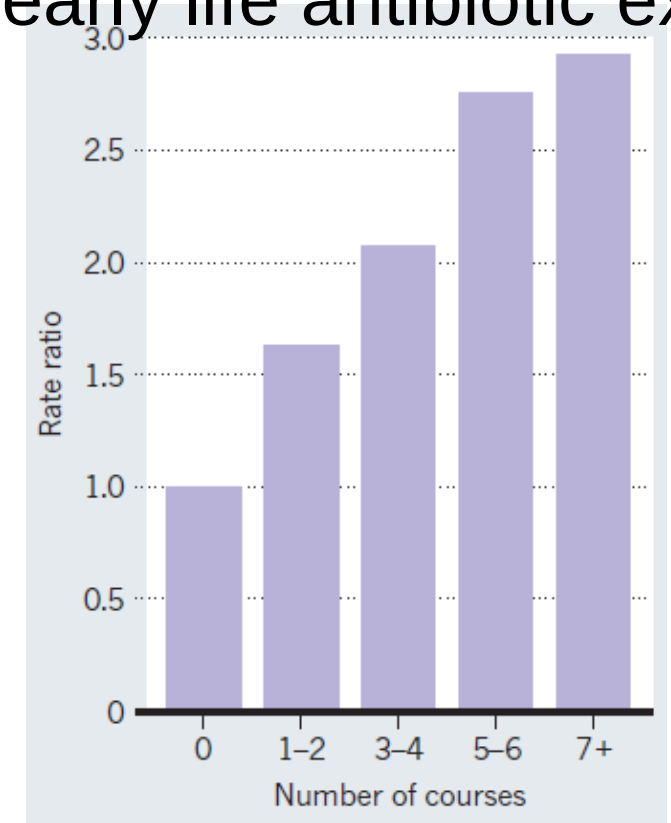


NOD Mice
Male
P12-23

Increased in PAT
Decreased in PAT

Mechanism

Likelihood of IBD in Danish children,
by early life antibiotic exposure



A. Hviid et al. *Gut* 2011; 60:49.

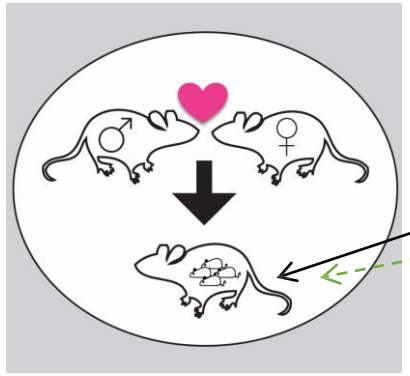
#4. IBD: Can an antibiotic-altered microbiota affect IBD outcome in the next generation?

C57BL/6 WT or IL10-/-

Spontaneous development of colitis

Does a perturbed microbiota have effects across generations?
Conventionalizing pregnant germ-free mice to examine effects on the offspring

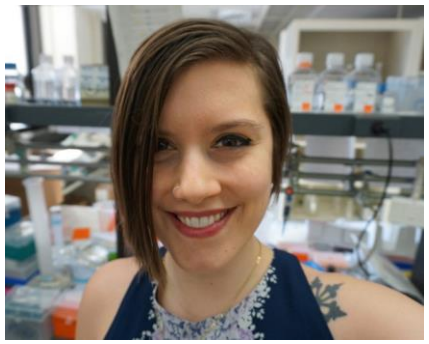
Germ-free



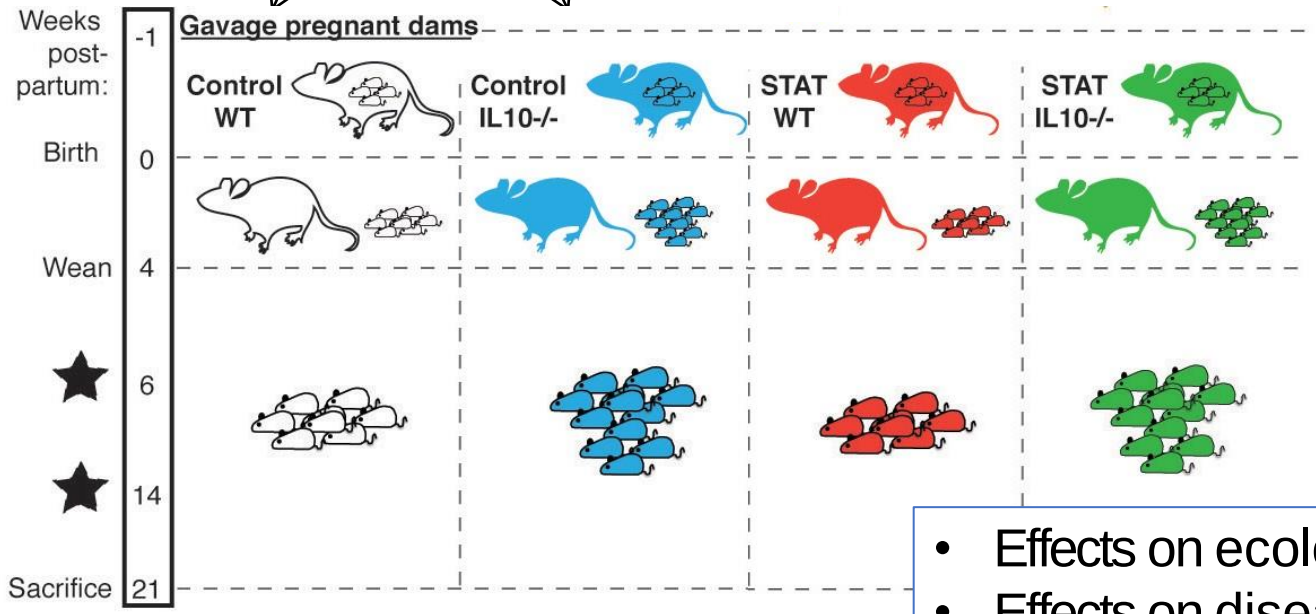
WT or IL10-/-

Control microbiota

Antibiotic-perturbed (STAT) microbiota

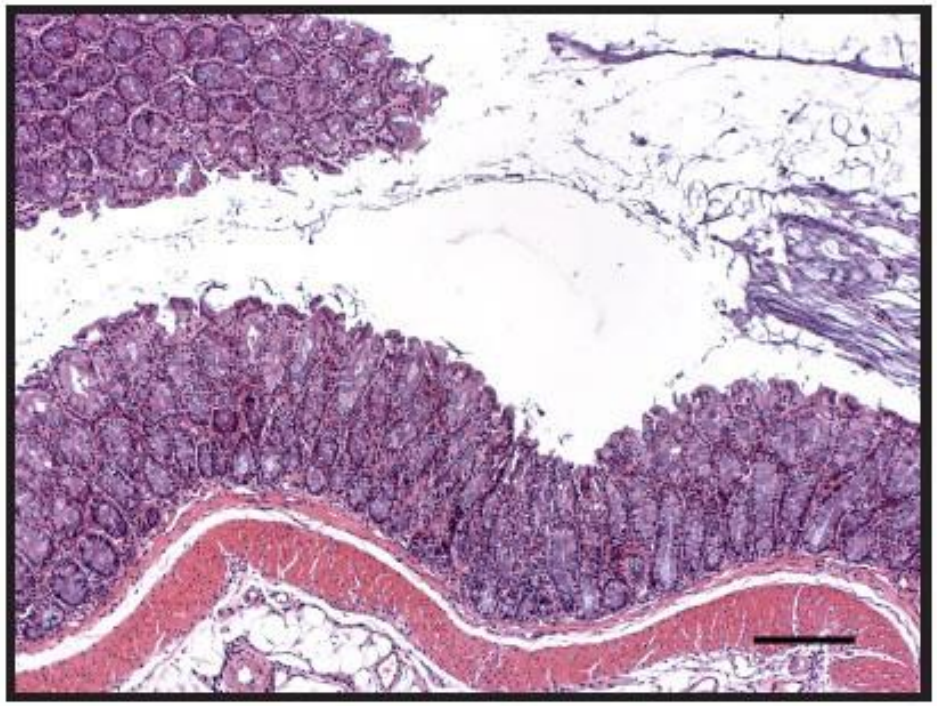


A Schulfer *et al*. *Nature Microbiology* 2017

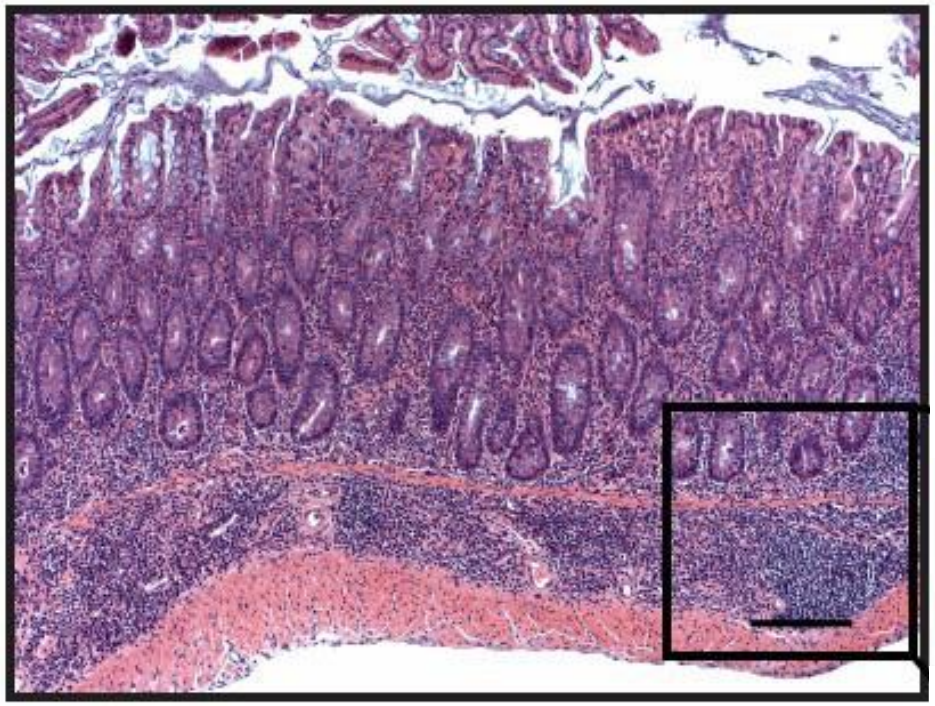


- Effects on ecology
- Effects on disease

Colonic pathology in IL-10-/- pups at week 21, according to the microbiota to which their mothers were exposed

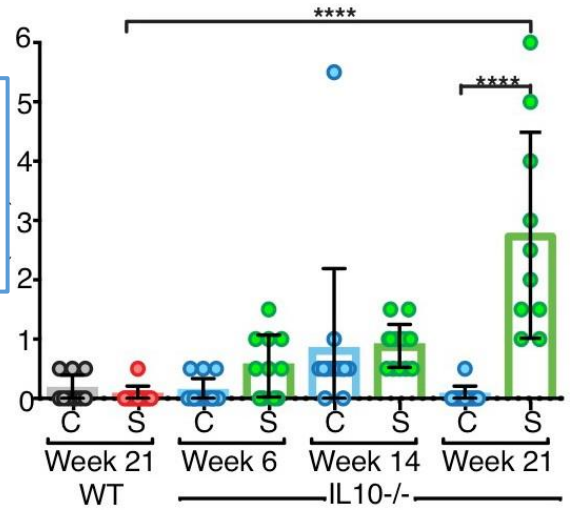


Control



Antibiotic-perturbed (STAT)

Histology Activity Index (HAI)



Summary

Neither pups nor mother received antibiotics

Enhanced disease signal is entirely microbial

Antibiotic effect crosses generations

Inheritance also based on microbial genes

Nature 2011:

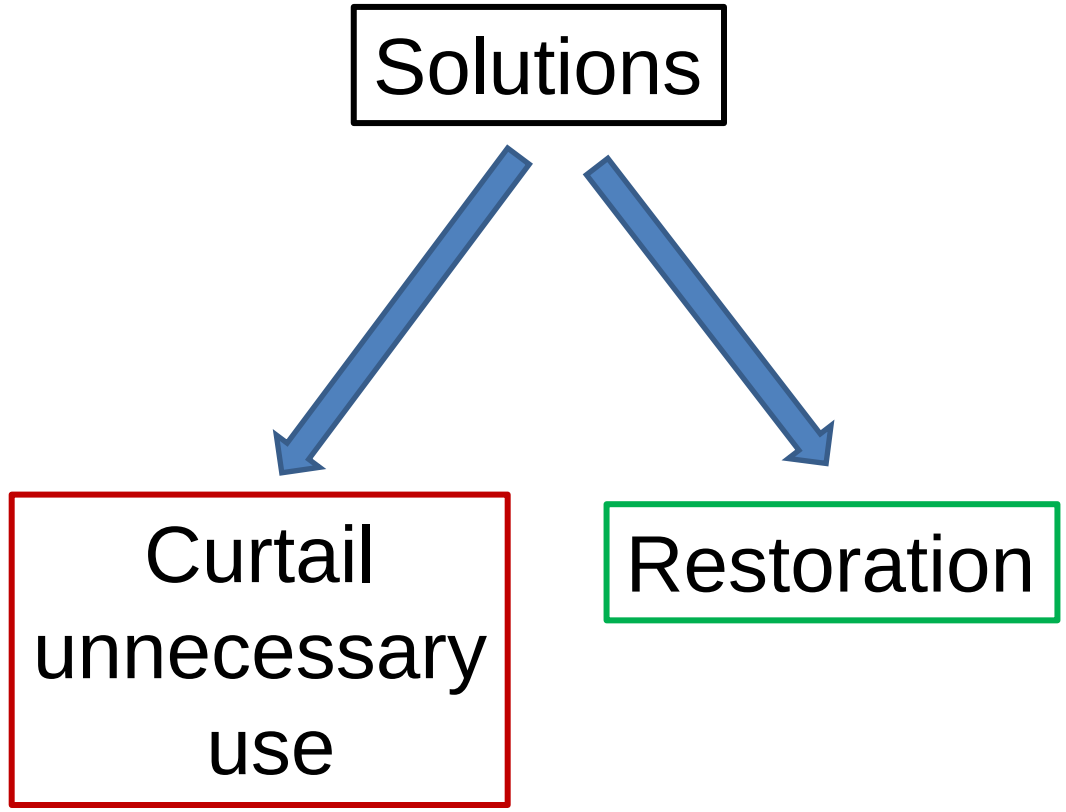
COMMENT

P. DUREUIL/PHOTOALTO/ALAMY

Dosed up: could excessive prescription of antibiotics be hampering children's ability to fight disease?

Stop the killing of beneficial bacteria

Concerns about antibiotics focus on bacterial resistance — but permanent changes to our protective flora could have more serious consequences, says **Martin Blaser**.



Cumulative antibiotic use in the USA, by age

Age (years)	Number of courses taken	
	USA	
	During period	Cumulative
2	2.73	2.73
3	-	-
10	8.17	10.90
20	6.78	17.68
40	13.38	31.06
65	19.93	50.98

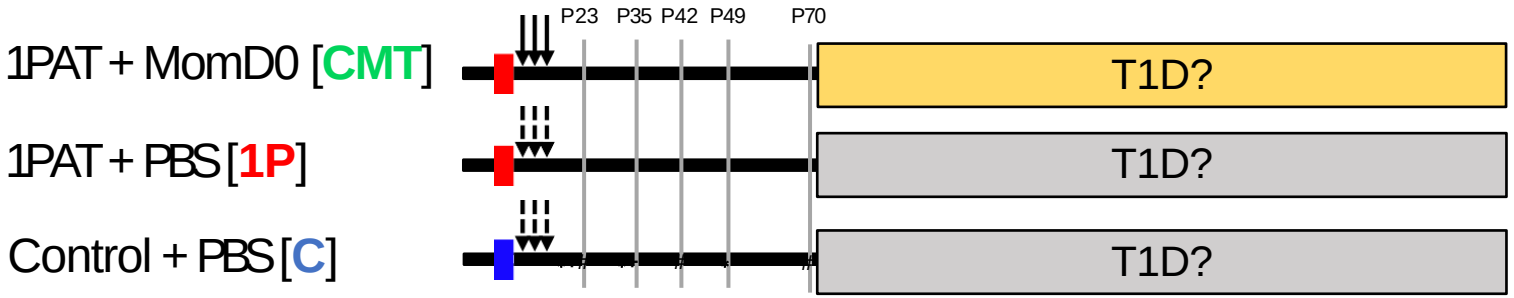
Adapted from L Hicks *et al NEJM* 2013; 368:1461

Cumulative antibiotic use in the USA and Sweden

Age (years)	Number of courses taken			
	USA		Sweden	
	During period	Cumulative	During period	Cumulative
2	2.73	2.73	-	-
3	-	-	1.39	1.39
10	8.17	10.90	2.90	4.28
20	6.78	17.68	2.52	6.80
40	13.38	31.06	5.92	12.72
65	19.93	50.98	8.48	21.20

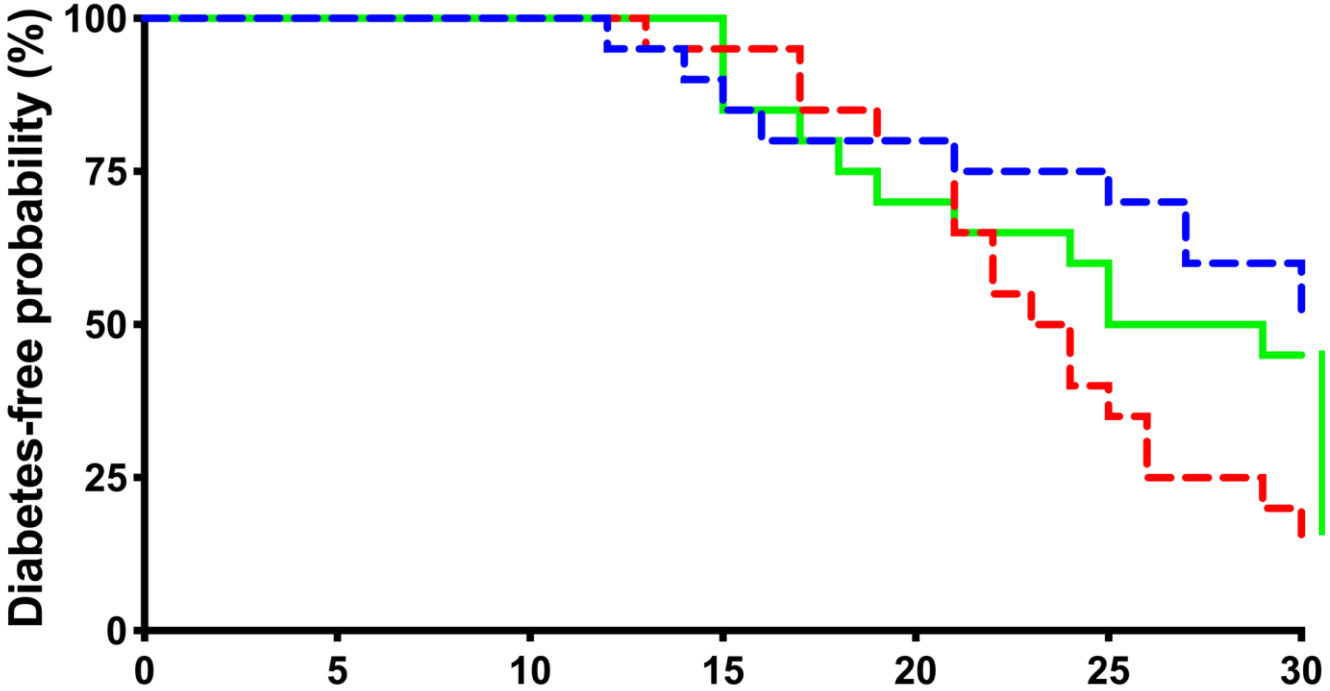
Adapted from L Hicks *et al. NEJM* 2013; 368: 1461 (USA)
A Ternhag *et al. NEJM* 2013; 369: 1175 (Sweden)

Reversibility: Effects of cecal microbiota transfer [CMT] on T1D development



Exposures, P5-9: Tylosin Non-acidified H_2O: Diabetes tracking from W12-W30

Gavages at P13, P15, and P17: MomD0 PBS



W30-survival
C 50%
1P 15%
CMT 45%

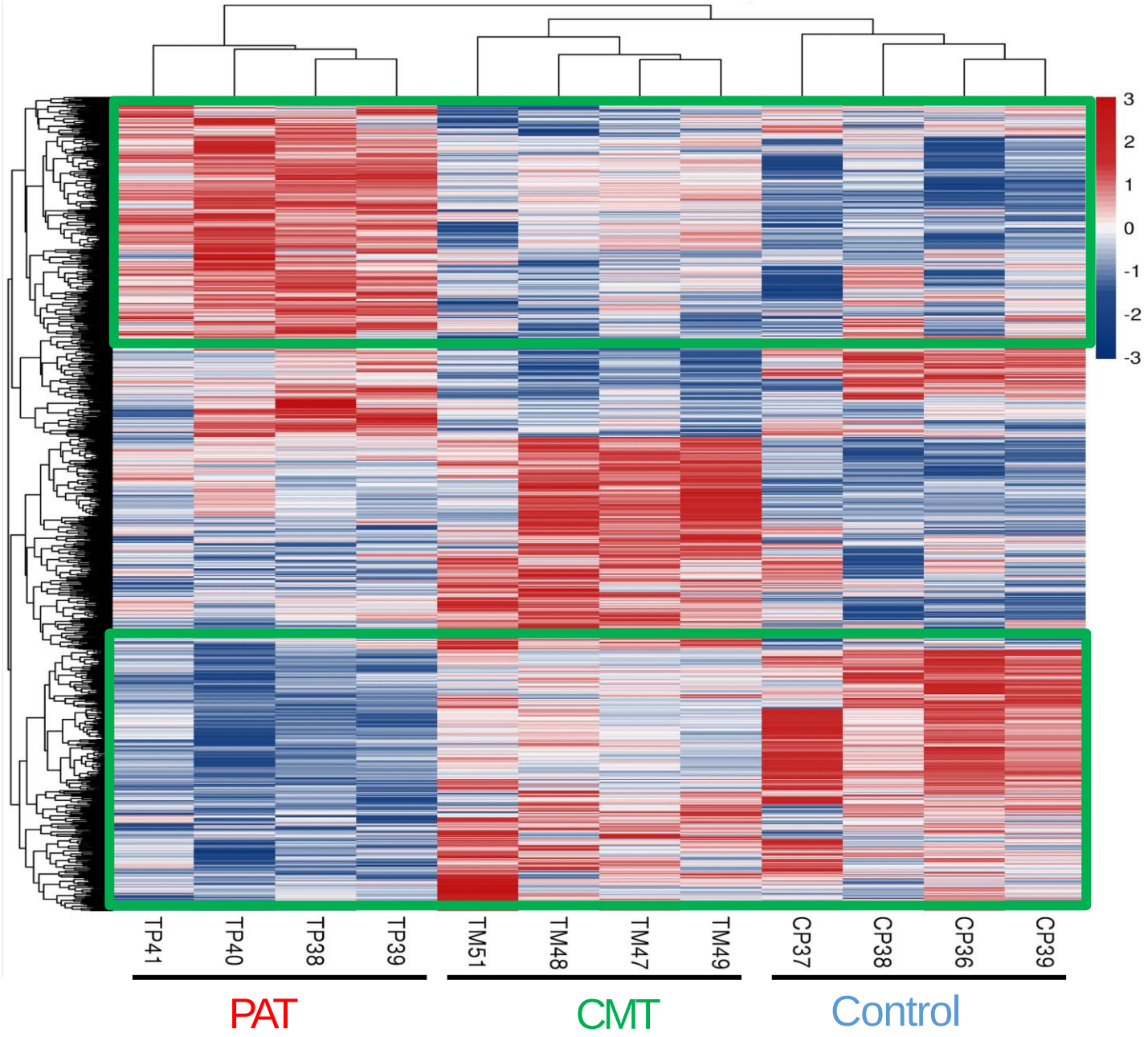
*p= 0.045

**p= 0.009

Xue-Song Zhang

X-S Zhang *et al.*
Cell Host &Microbe
2021

Mechanism: differential ileal gene expression in relation to CMT restoration



Method: RNAseq
Timing: P23
Groups: Control
PAT
Restored CMT
Analysis: Unsupervised hierarchical clustering

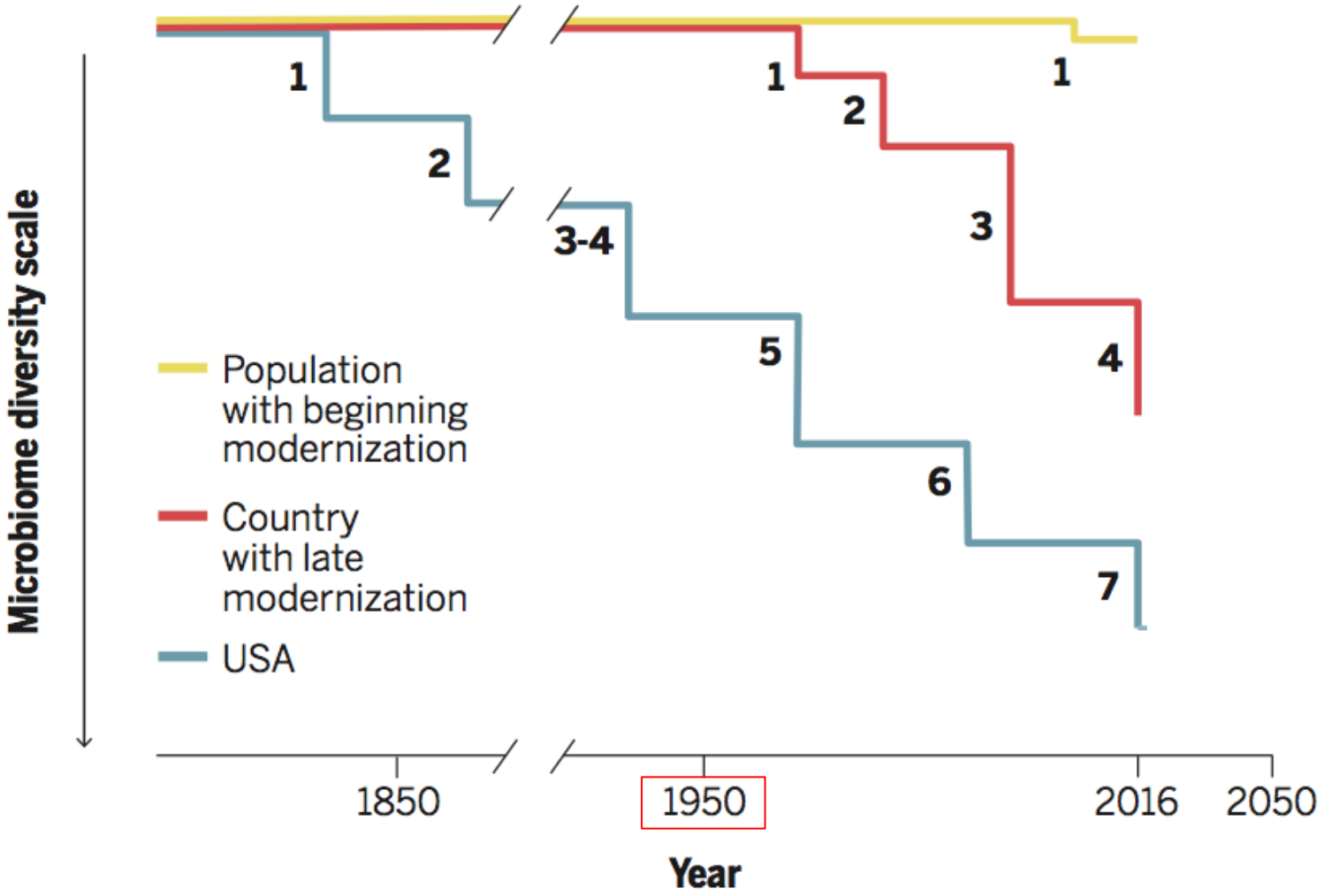
Pathway for discovery:
Microbes
Microbial genes
Metabolites
Host genes
perturbed by antibiotics that drive altered development and that can be restored

X-S Zhang *et al. Cell Host &Microbe* 2021

Bradford Hill criteria: for establishing evidence of a causal relationship between a presumed cause (antibiotic exposure) and an observed effect (obesity development)

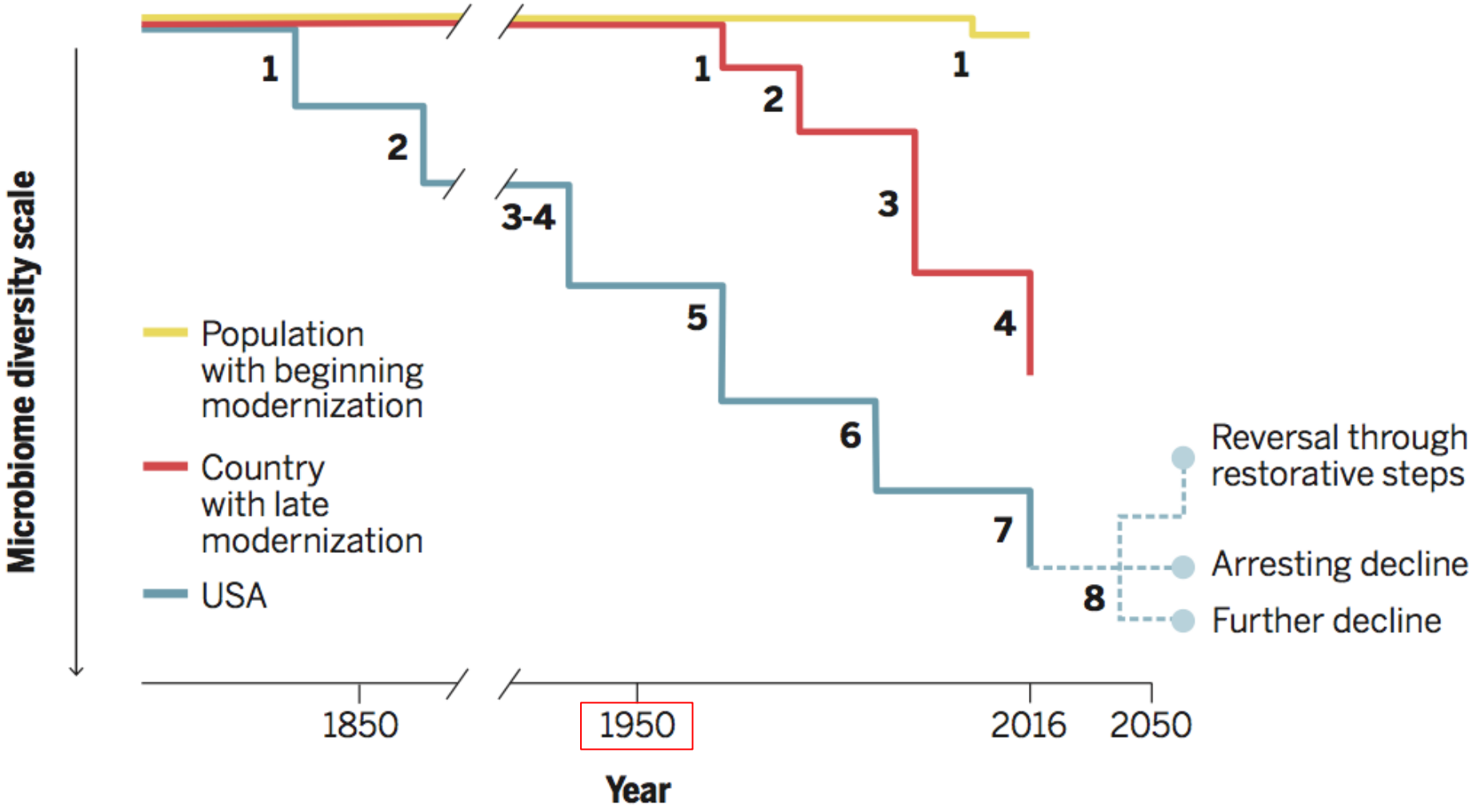
Strength	Odds ratios: 1.10-1.60
Consistency	Multiple independent studies
Specificity	Associations with multiple other conditions
Temporality	Yes, in multiple studies
Biological gradient	Geographic studies; dose response
Plausibility	Consistent with known roles of the microbiome in host metabolism
Coherence	Concordance between epidemiological and laboratory findings [Similarity with parallel relationship: C-section and obesity]
Experiment	Multiple
Analogy	Growth promotion using antibiotics: practiced for 75 years
---	
Reversibility	Not yet shown, but evidence with asthma and T1D

Diversity loss in the microbiome in 3 model locales

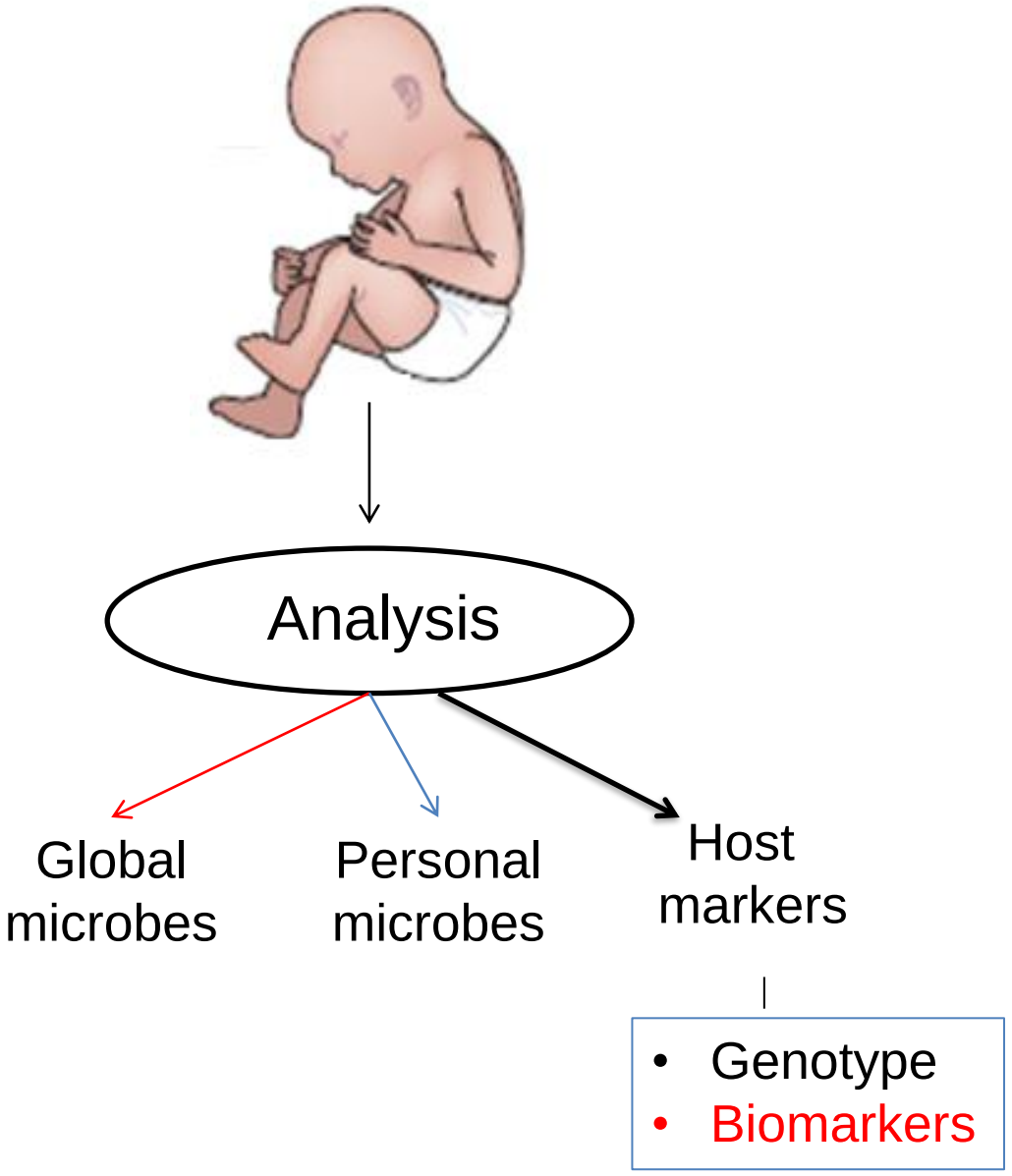


Science 2016

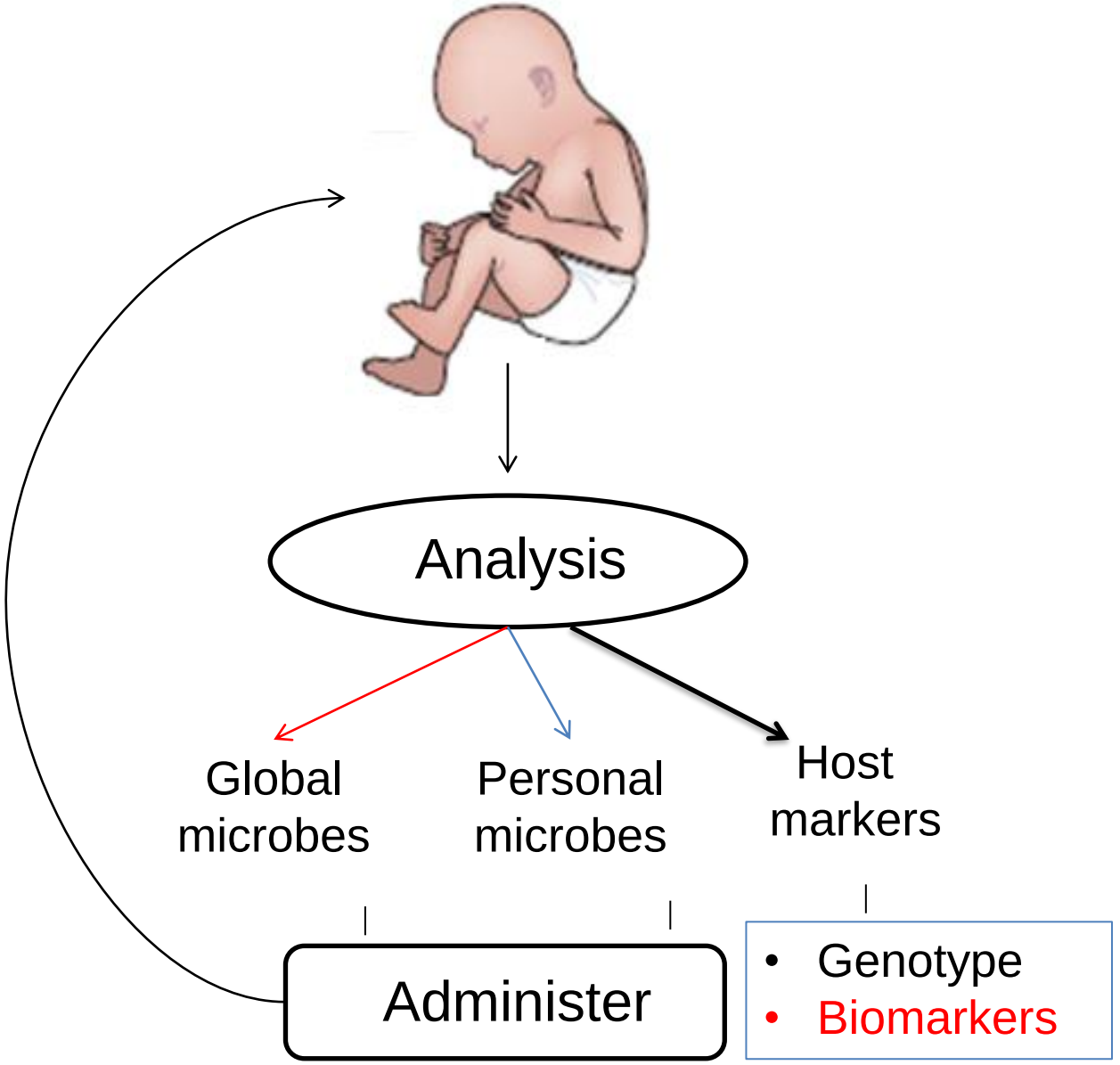
Next steps for the microbiome?



Medicine of the future: new analyses for obesity prevention



New approach to prevent obesity?



New algorithm for preventing obesity?