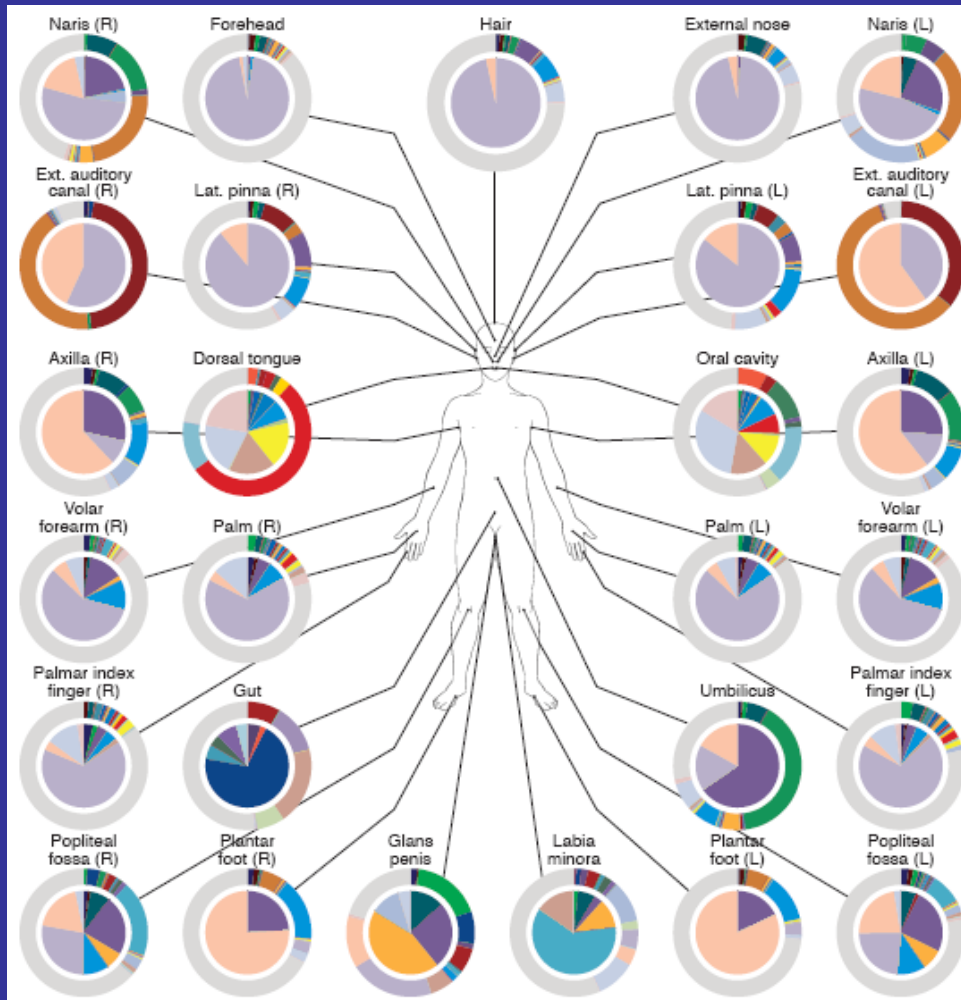


# You are what they eat: the human gut microbiome in nutrition and health

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# Microbes dominate the human body



- You are half human and half microbe – the number of microbes are roughly the same as human cells
- Microbes colonize almost every body environment
- Each habitat on an individual has a different microbiome
- Human beings are a biosystem

# 1. Introduction to metagenomics

How do we know...

- who's there
- what they are doing
- why they are important

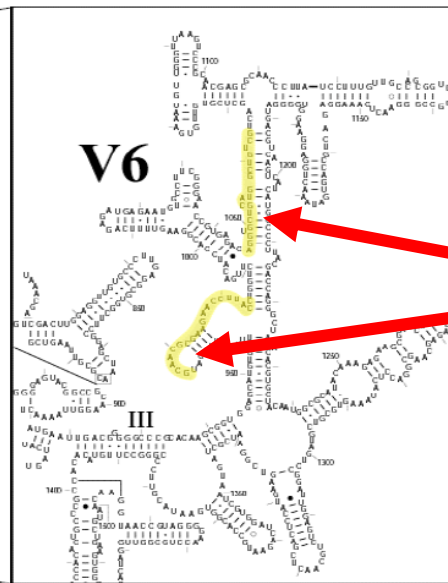
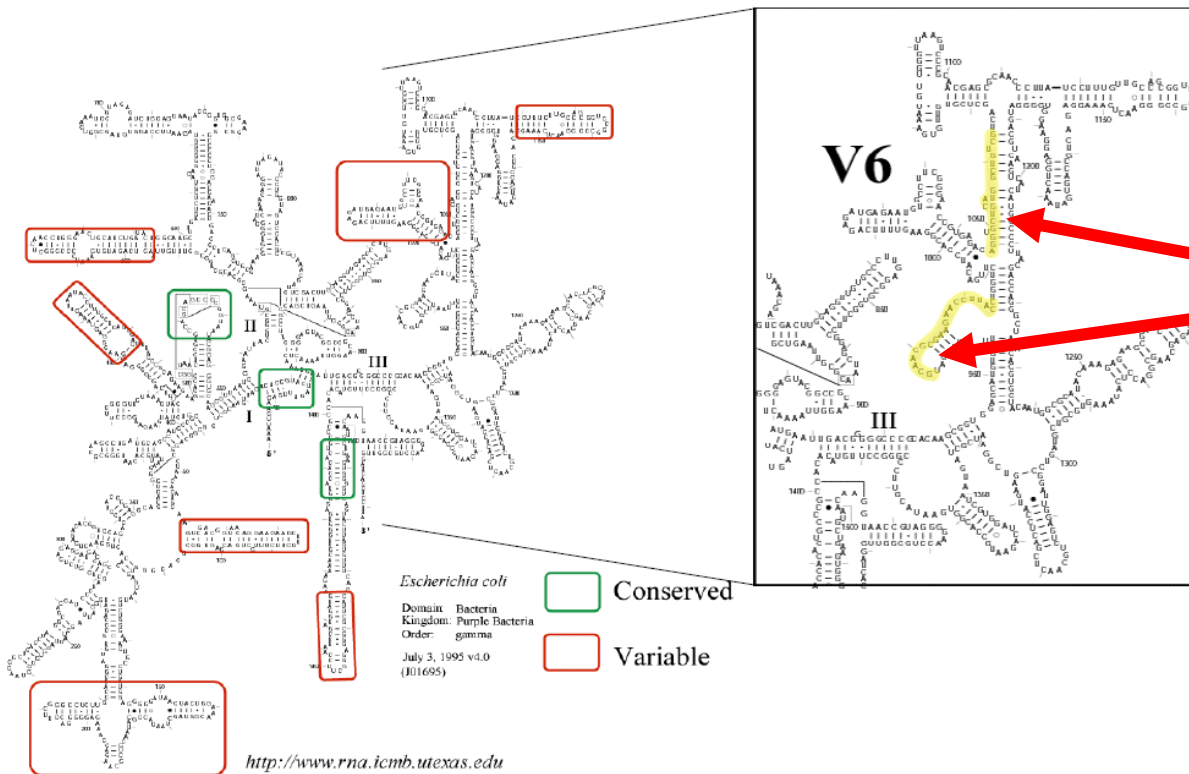
# 2. The role of the human gut microbiome

- What are functions of our bacterial symbionts?
- Where does the gut microbiome come from?
- How do the gut microbiome and nutrition interact to influence health?
- What happens when the microbiome is disrupted?

# How do we know who's there?

Microbiome = community with many members  
Need a unique id for every member

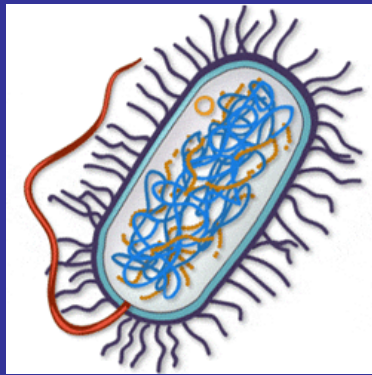
## Small subunit ribosomal RNA



Hypervariable  
Regions

# How do we know who's there?

## 16S rRNA Gene: a bacterial barcode



Primer and barcode



16S

Hypervariable Region

Primer



16S

Amplify and Sequence

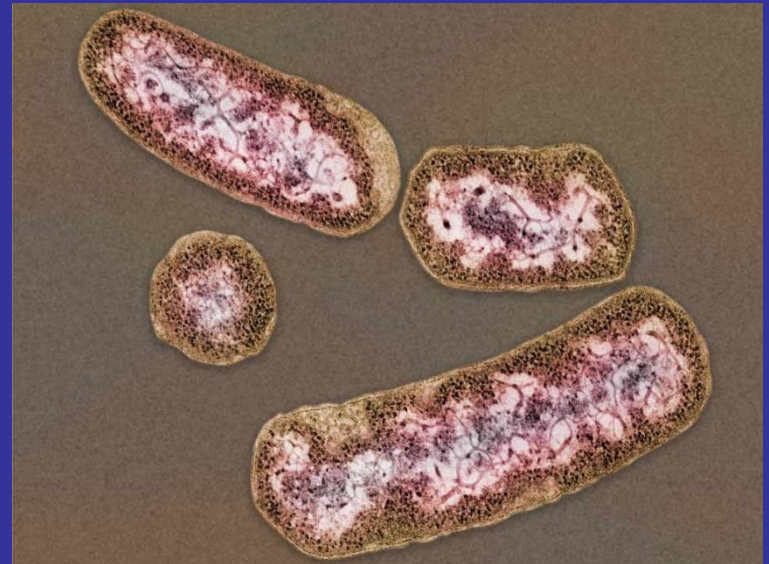
# Who's there

>G70SE5B01A76Y7

```
GGACCGTGTCTCAGTTCCAATGTGGGGGACCTTCCTCTCAGAACCCCTACT
GATCGTTGCCTTGGTGGGCCGTTACCCCGCCAACAAGCTAATCAGACGCAT
CCCCATCCATCACCGATAAATCTTTAATCTCTTTCAGATGTCTTCTAGAGATAT
CATTGGGTATTAGTCTTACTTTCGCAAGGTTATCCCCAAGTGGTGGGCAGGT
TGGATACGCGTTACTCACCCGTGCGCCGGTCGACGCCTATCGGAAGCAAGC
TTCCAATATCGTTTCCCCTCG
```

## Prevotella

- Normal GI bacterium
- Also in mouth
- Some types cause infection





# “Omics” methods for bacteria

- Genomics – sequencing and assembling the entire genome of an organism
- Metagenomics – genomics across microbiome to identify gene abundance across as proxy for function
- Metatranscriptomics – actual gene activity identified from transcripts
- Proteomics – characterization of the proteins produced by the microbiome
- Metabolomics and Metabonomics – identification of bacterially-derived metabolites in host

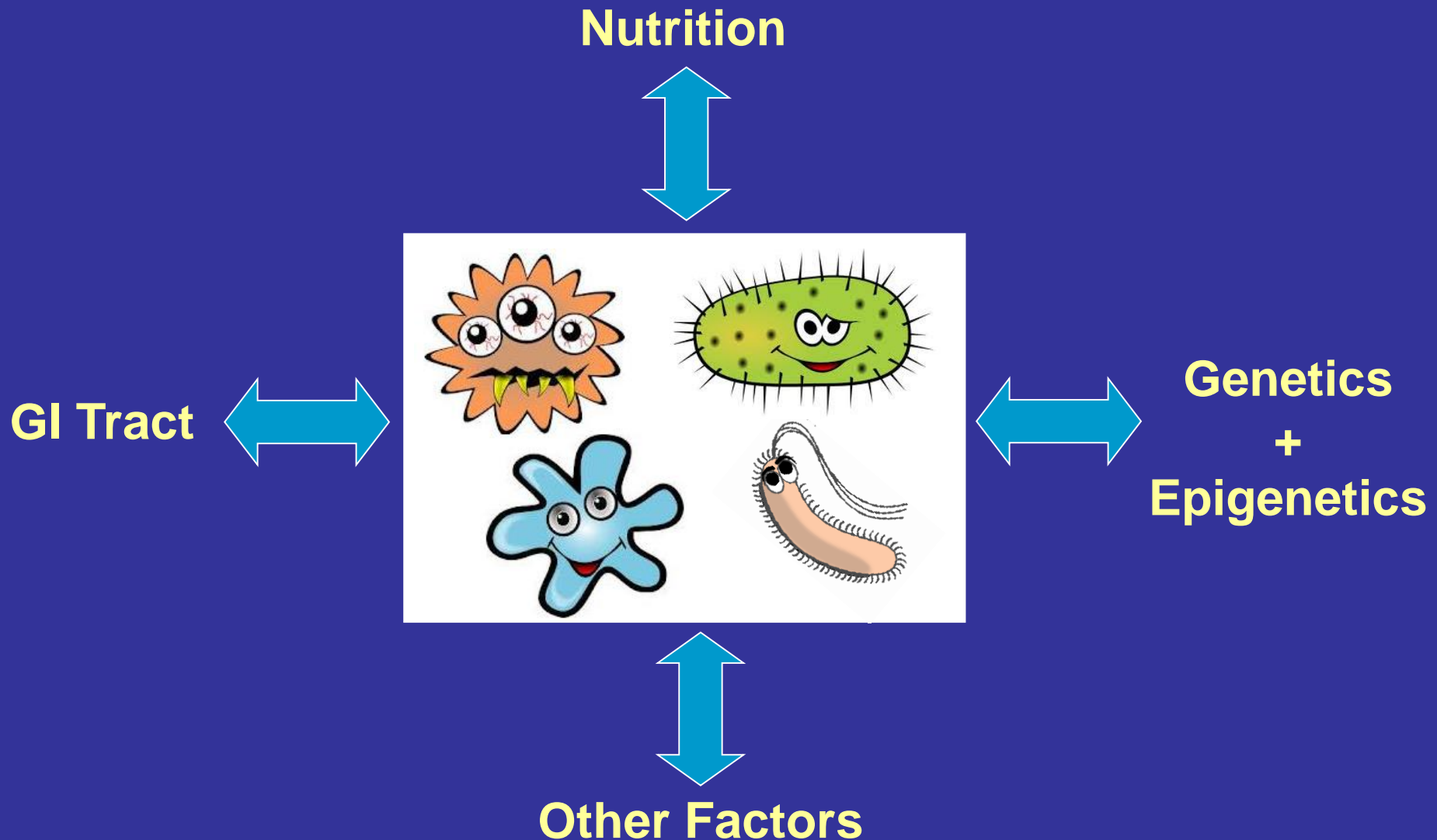


## 2. The role of the human gut microbiome

- Why are gut microbes important?
- Where does the gut microbiome come from?
- How do the gut microbiome and nutrition interact to influence health?



# A complicated set of interactions

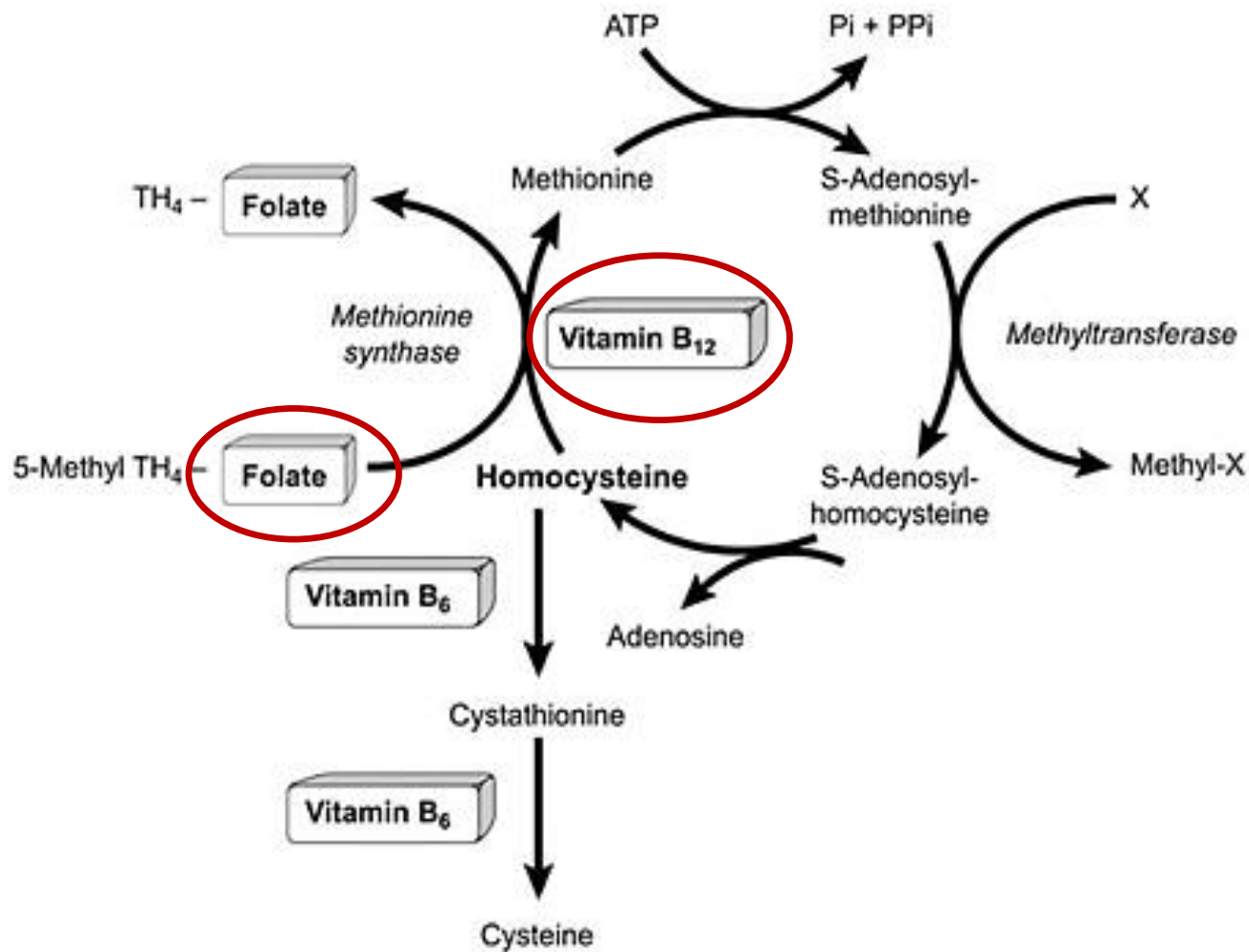


# Some gut microbiome functions

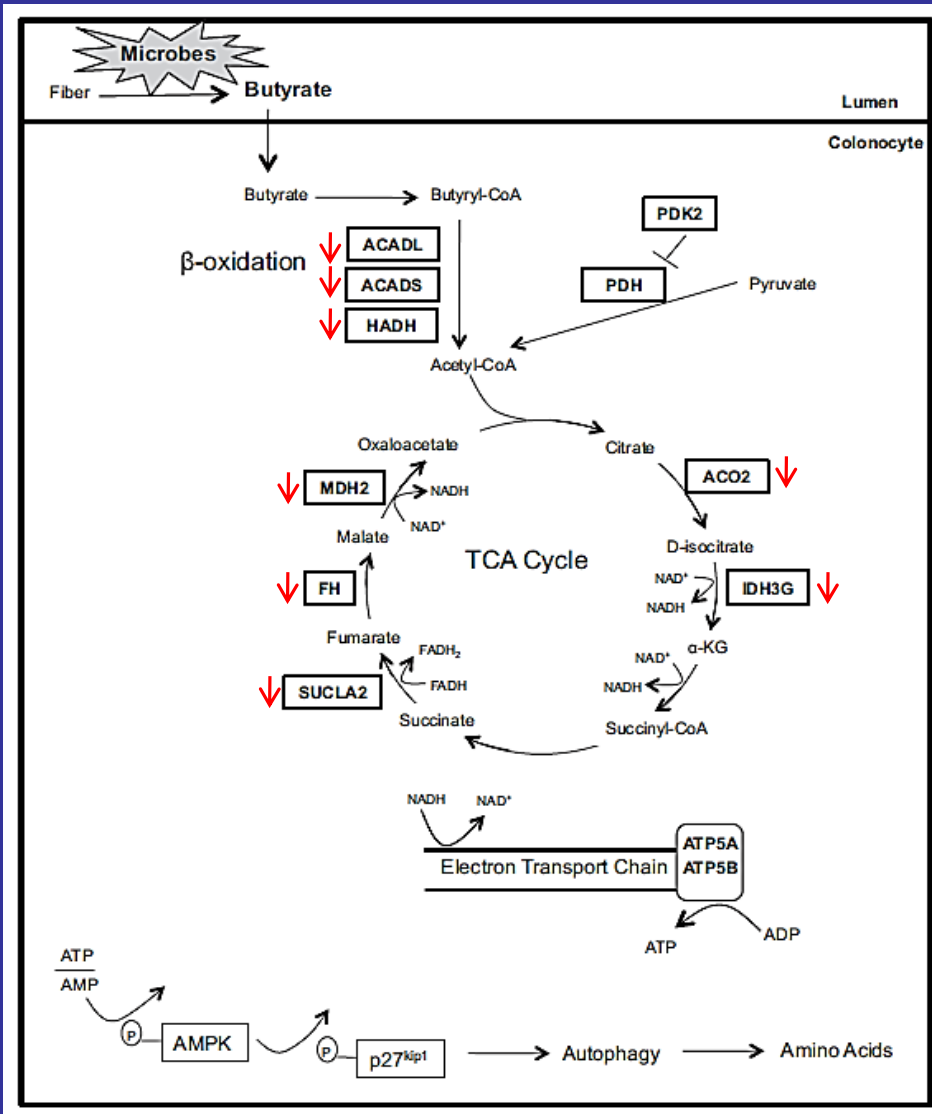
- Processing nutrients
- Synthesizing micronutrients
- Digesting the indigestible
- Harvesting energy
- Maintaining redox balance in the intestine
- Promoting intestinal health
- Tuning the developing immune system
- Directly inhibiting pathogens

Gut microbiome adapts and responds to host and external changes

# Bacteria-mediated nutrients in homocysteine metabolism



# Nutrition/microbe interactions in the colon



Microbiome-produced butyrate provides the primary energy source for colonocytes.

Microbiota maintain NADH/NAD<sup>+</sup> ratios and ATP levels in the colon.

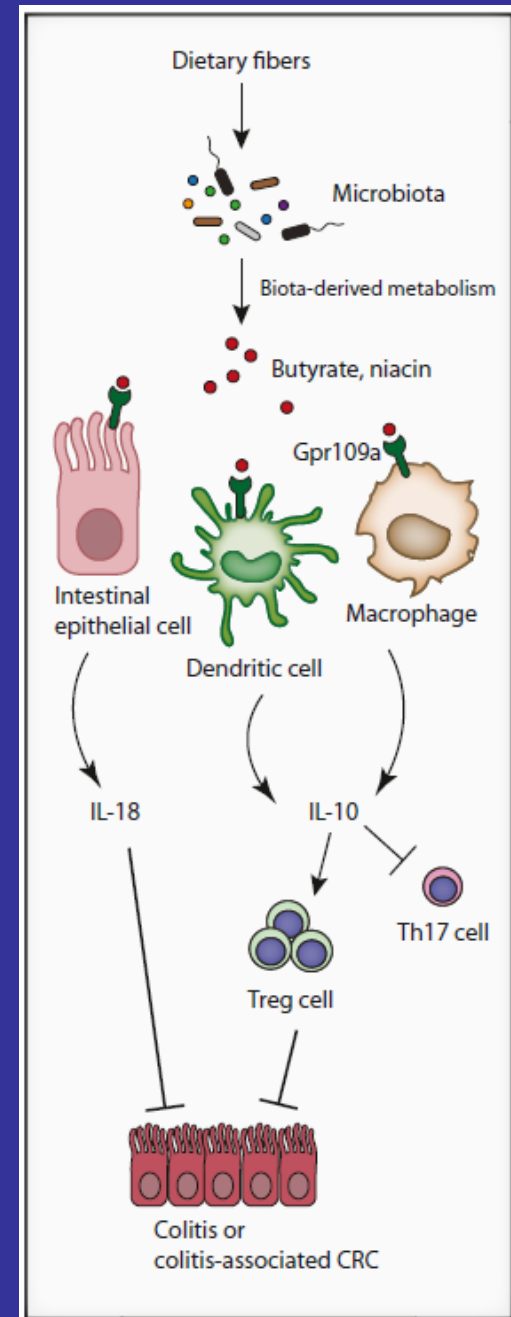
Germ-free mice have greatly reduced colonocyte ATP levels.

TCA cycle enzymes (boxed) are regulated by gut microbes.

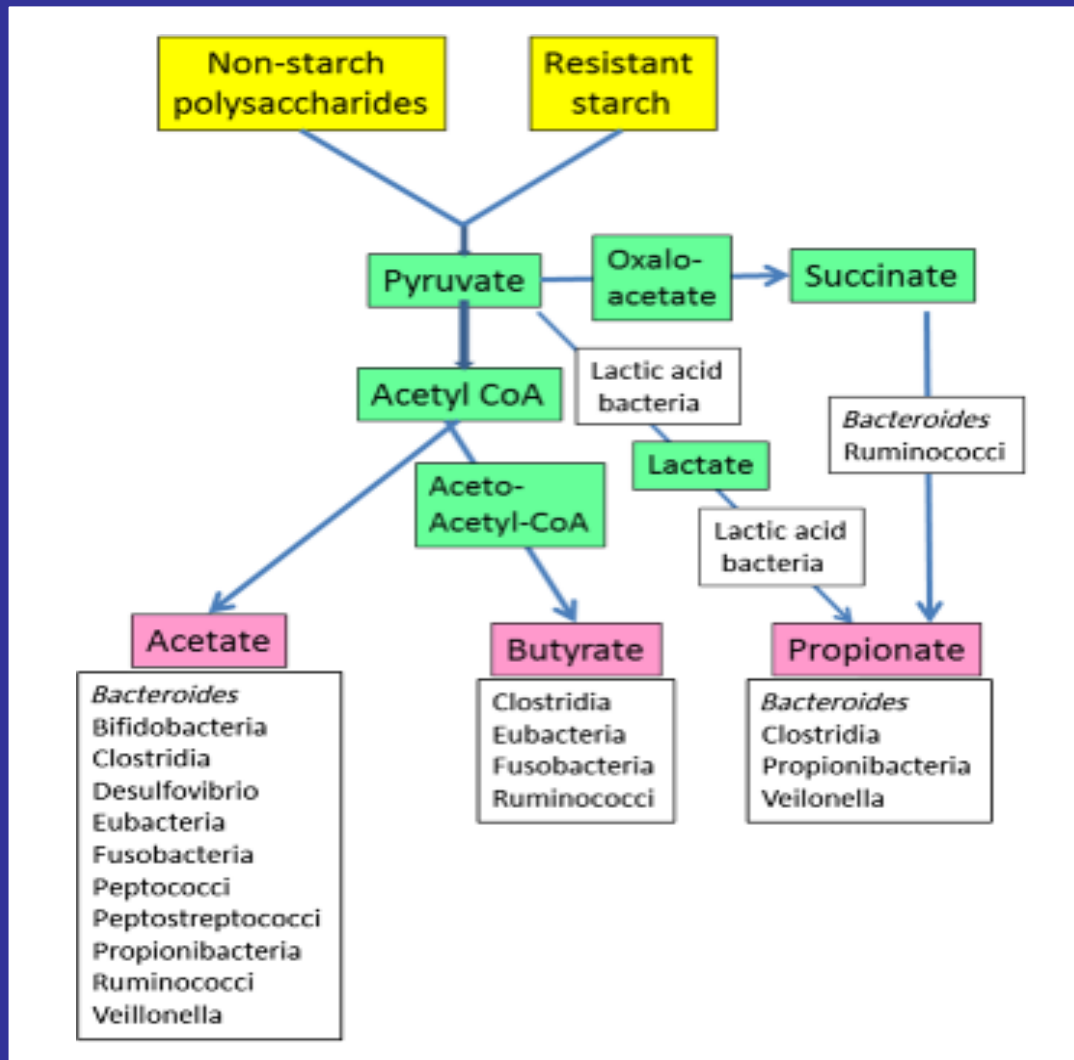
Enzymes downregulated in germ-free mice are indicated (↓).

# Diet, Microbiome and Gut Health

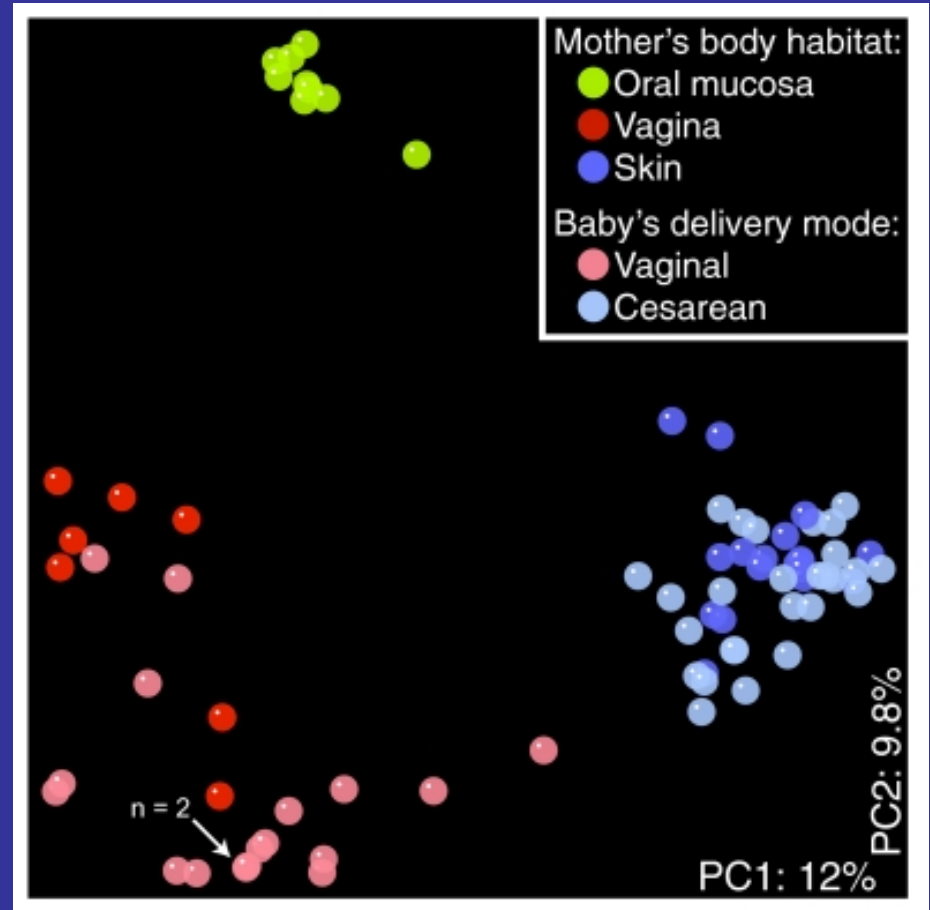
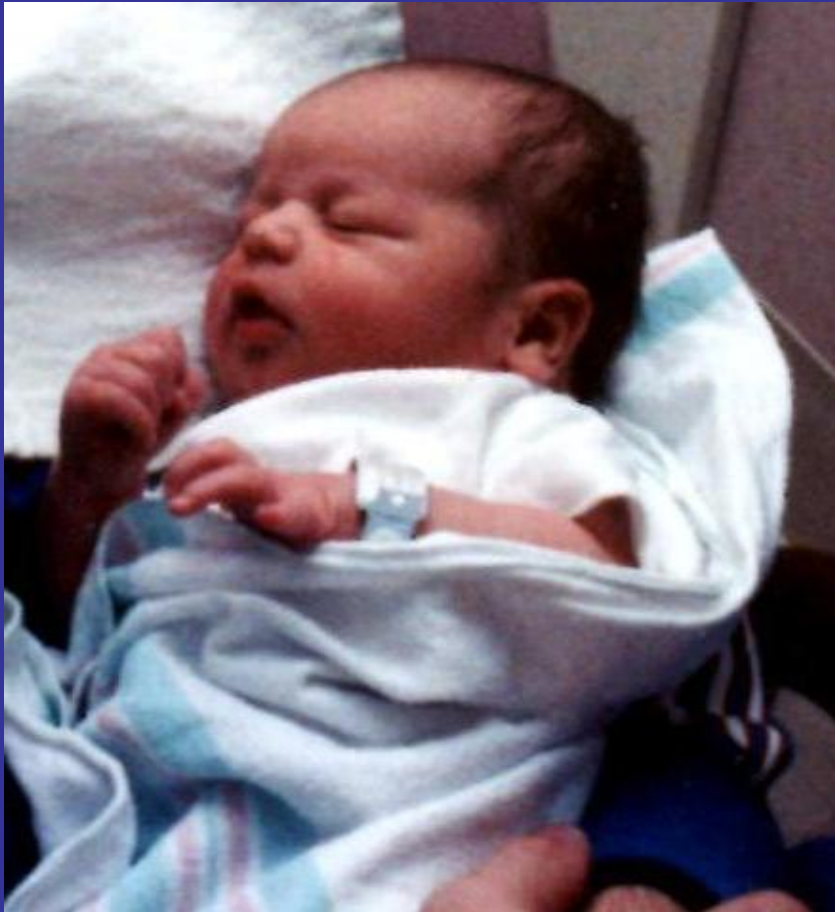
- Metabolites are generated from dietary fiber by gut bacteria
- Butyrate and niacin are ligands for G protein-coupled receptors on intestinal and immune cells
- Activated receptors trigger the production of agents that are protective and regulate the immune system
- Lack of dietary fiber affects Th1 and Th17 responses in autoimmune conditions



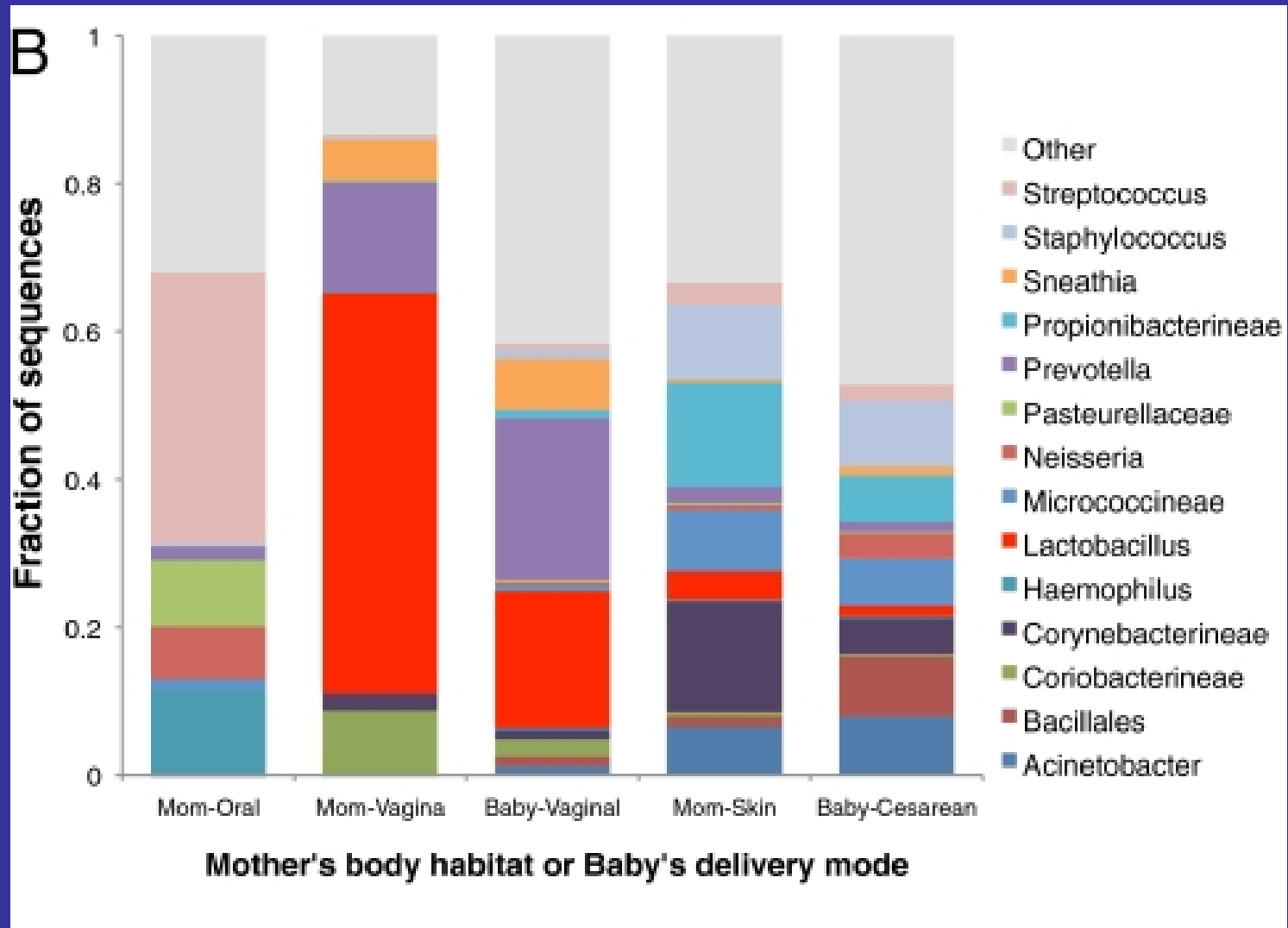
# Bacteria that produce short-chain fatty acids



# Acquiring a gut microbiome



# Initial gut microbiome depends on delivery method





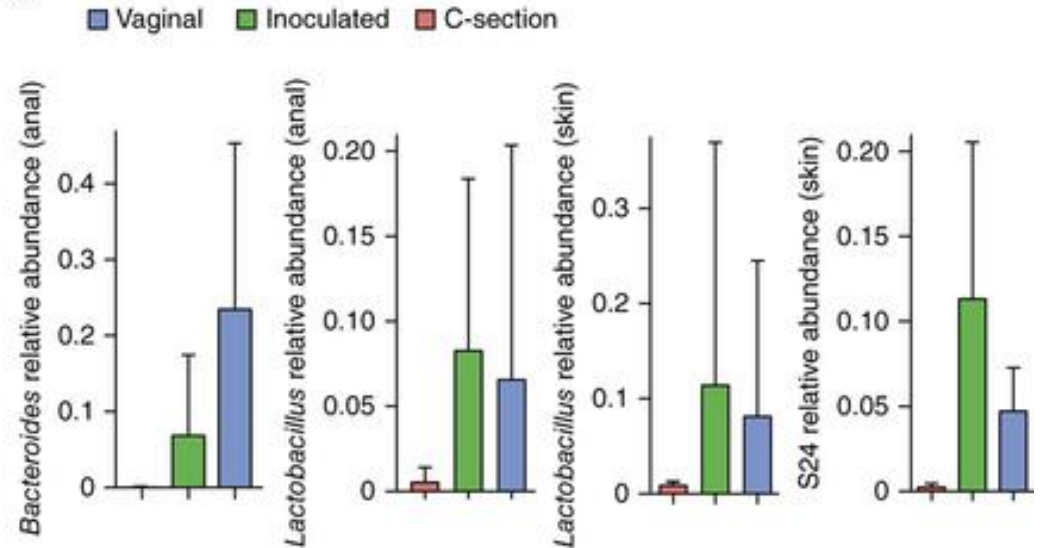
# Restoration of Gut Microbiome

**a**



Image: M.J. Schoen

**d**

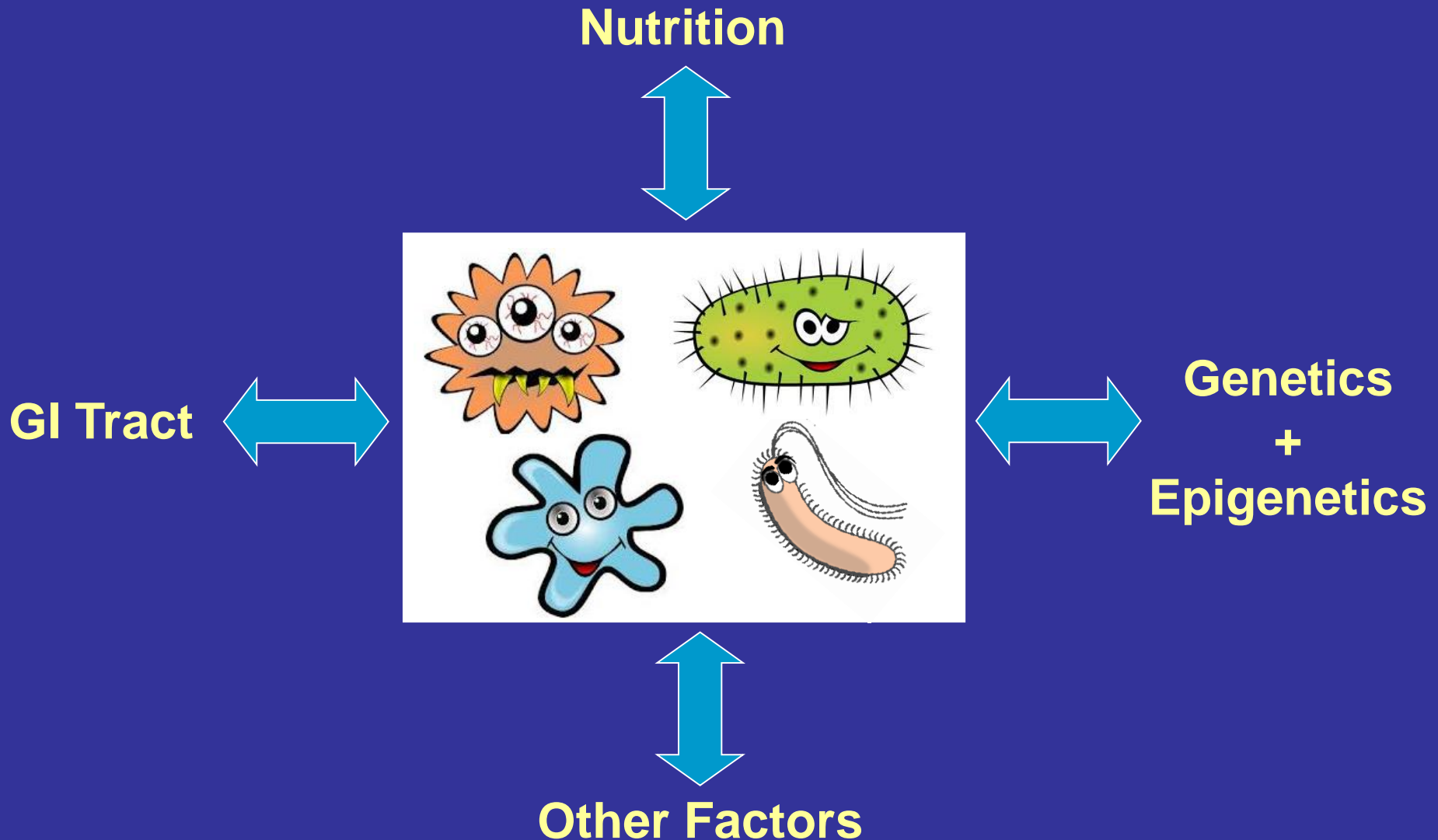


Inoculation of vaginal microbiome partially restores C-section baby gut microbiome

# The role of the human gut microbiome: Conclusions

- Gut microbes and human hosts in functional symbiosis.
- Relationships among gut microbes, nutrition, the gastrointestinal tract, human genetics and other host factors are complex and often interdependent.
- They participate in human metabolism, synthesizing nutrients, such as tetrahydrofolate and vitamin B12, integral to metabolic processes.
- Gut microbes produce produce short-chain fatty acids, including butyrate which is the primary energy source for colonocytes and regulates  $\beta$ -oxidation and the TCA cycle in the colon.
- The gut microbiome is acquired both before and at birth and is initially different from the adult microbiome.
- The types of microbes that initially colonize an infant are affected by whether birth is vaginal or cesarean.

# Diet, the gut microbiome and health



# How much does diet affect the microbiome?



15 Children in  
Florence, Italy



14 Children in  
Burkino Faso



# Do different diets = different microbiota?

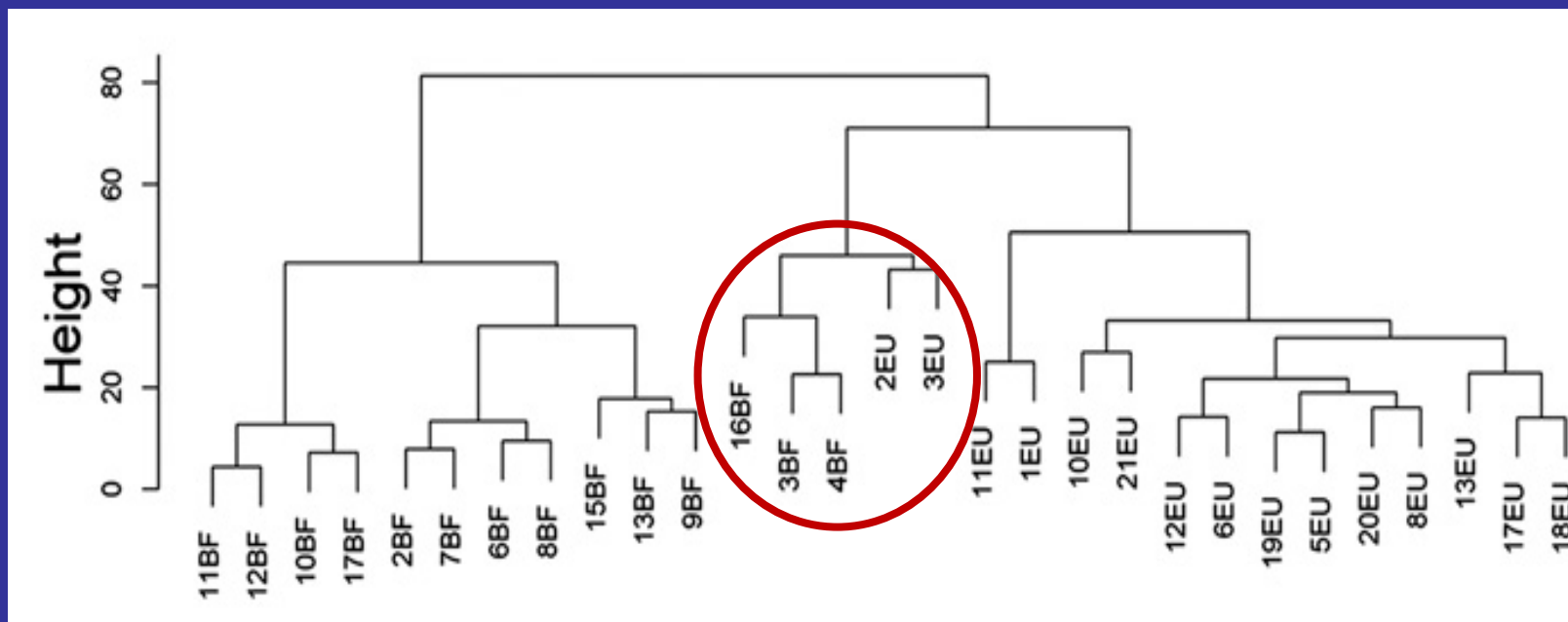
## Florence, Italy (N=15)

- Western diet
- Low in fiber
- Rich in animal protein, sugar, starch and fat
- Processed foods
- Breast fed up to 1 year
- Calories:
  - 1-2 yr = 1069 kcal
  - 2-6 yr = 1517 kcal

## Burkina Faso (N=14)

- Traditional rural African diet
- Predominantly cereals, legumes and vegetables
- Low in fat, animal protein
- Rich in starch, fiber, plant polysaccharides
- Cultivated and harvested locally
- Breast-fed up to 2 years
- Calories:
  - 1-2 yr = 672 kcal
  - 2-6 yr = 996 kcal

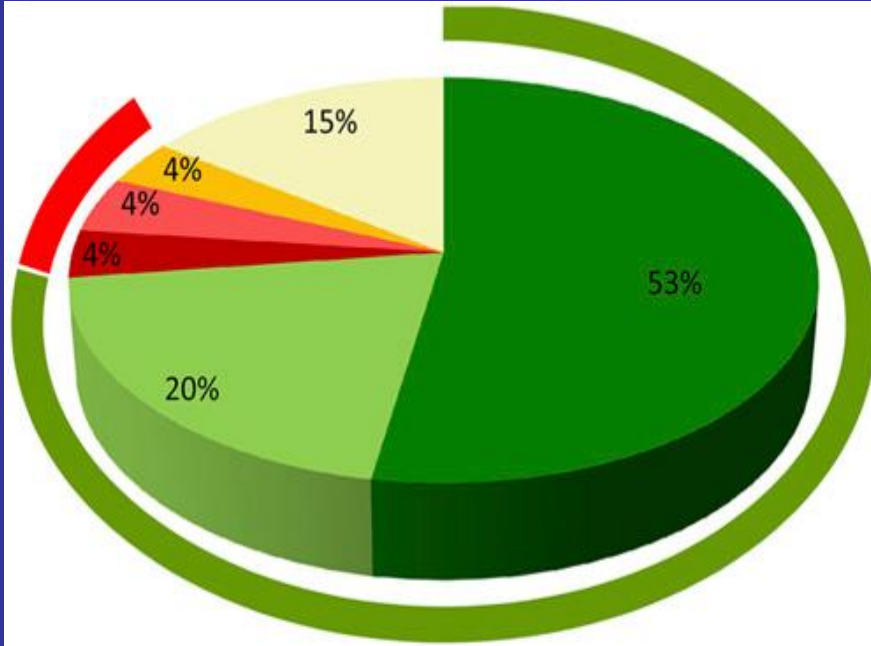
# Clustering of samples from BF and EU children



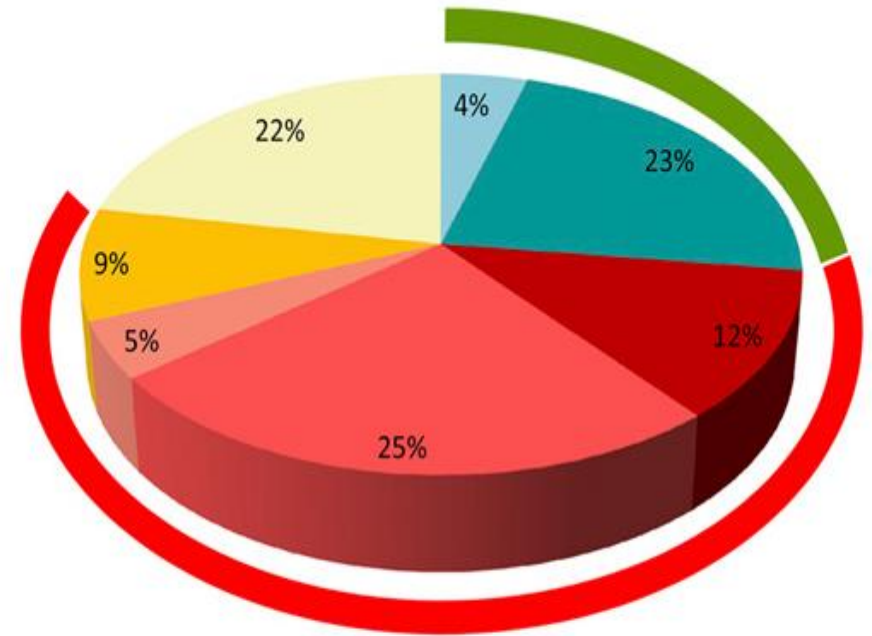
- Clusters generally separate by group
- Cluster between groups includes young, breast-fed children from both populations
- Similar microbial communities of young children on breast milk diet



# Diet shapes who is there

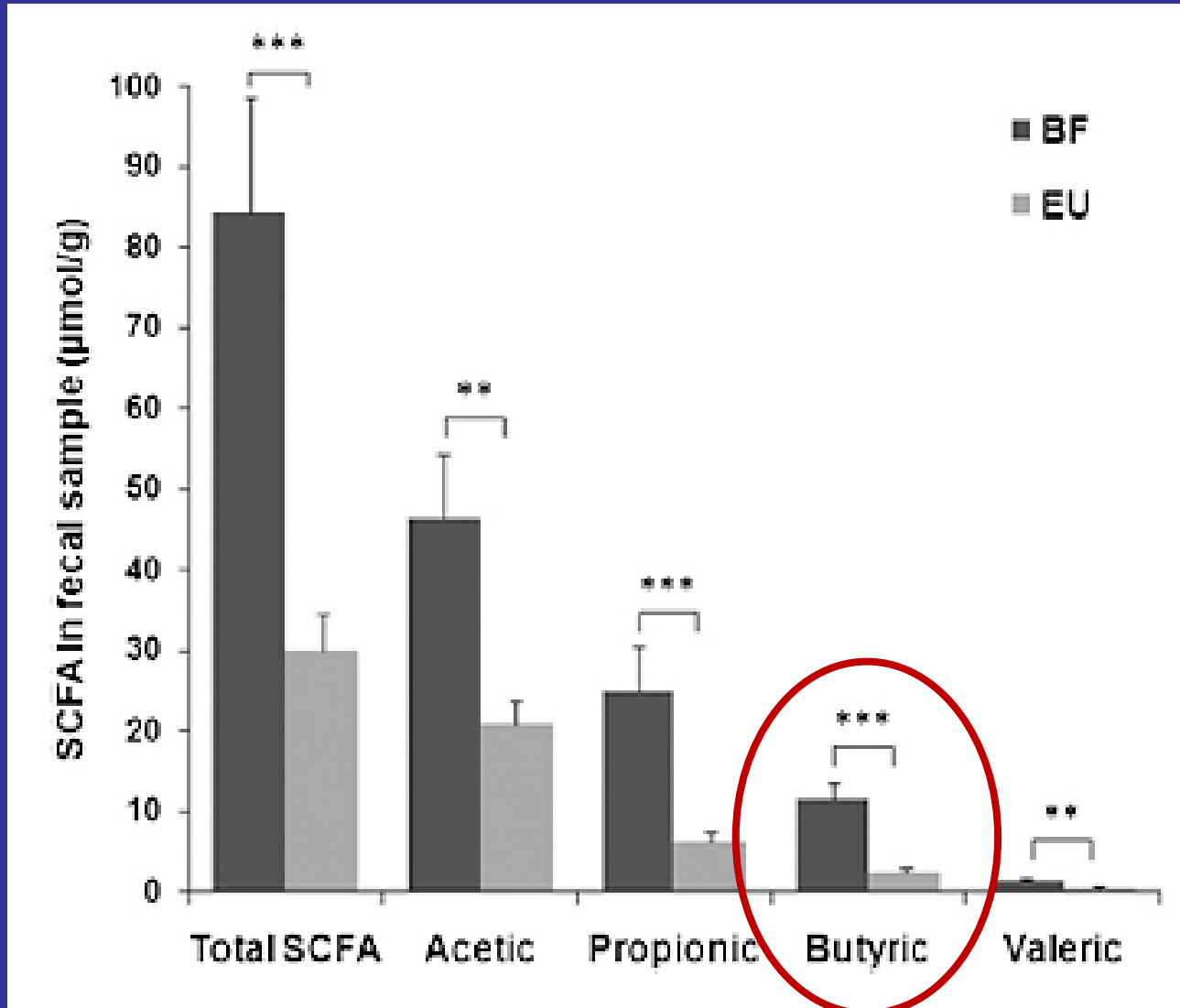


- BF**
- Prevotella } Bacteroidetes
  - Xylanibacter } Bacteroidetes
  - Acetitomaculum } Firmicutes
  - Faecalibacterium } Firmicutes
  - Subdoligranulum } Firmicutes
  - Others



- EU**
- Alistipes } Bacteroidetes
  - Bacteroides } Bacteroidetes
  - Acetitomaculum } Firmicutes
  - Faecalibacterium } Firmicutes
  - Roseburia } Firmicutes
  - Subdoligranulum } Firmicutes
  - Others

# Diet shapes what they do

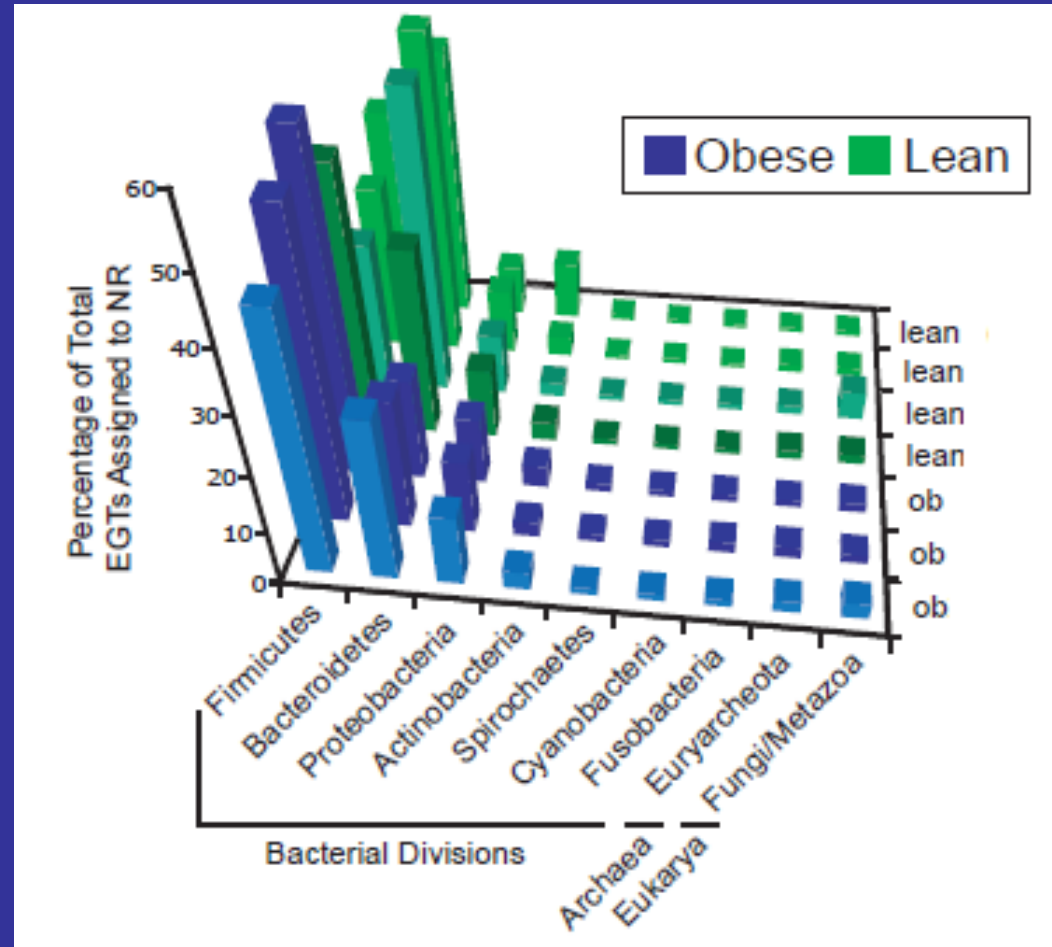




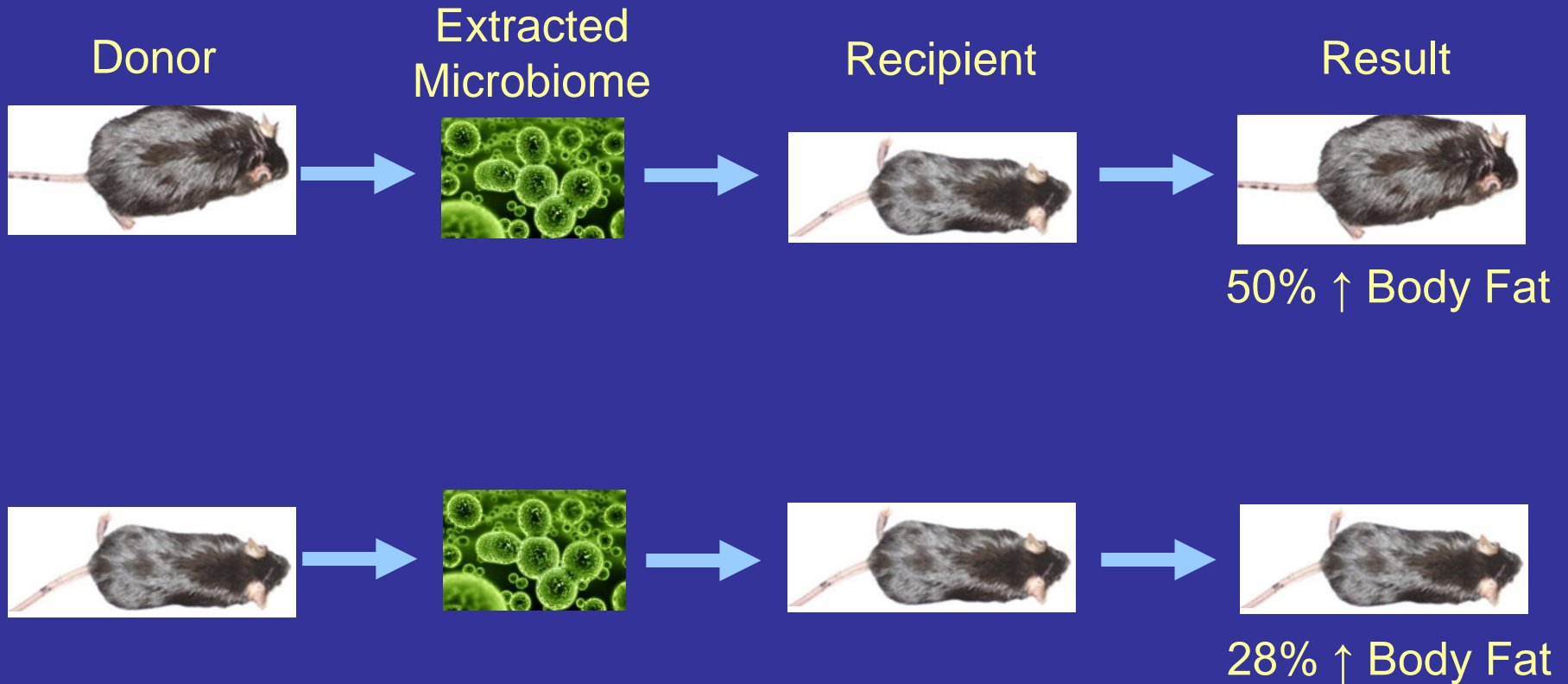
# Genetics & the gut microbiome in obesity



<http://www.sciencedaily.com/images/2007/01/070129081348.jpg>



# Determining cause and effect

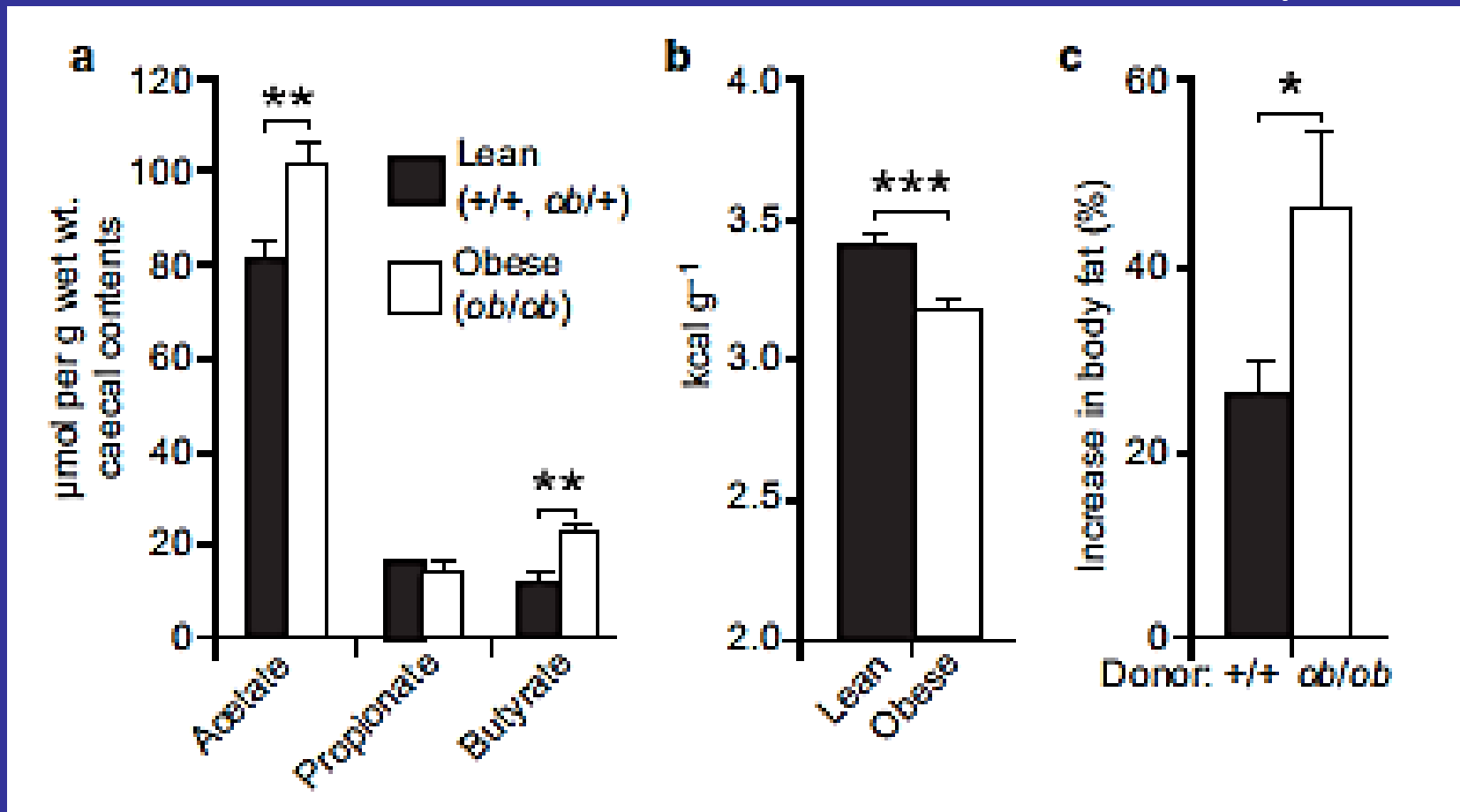


# Determining cause and effect

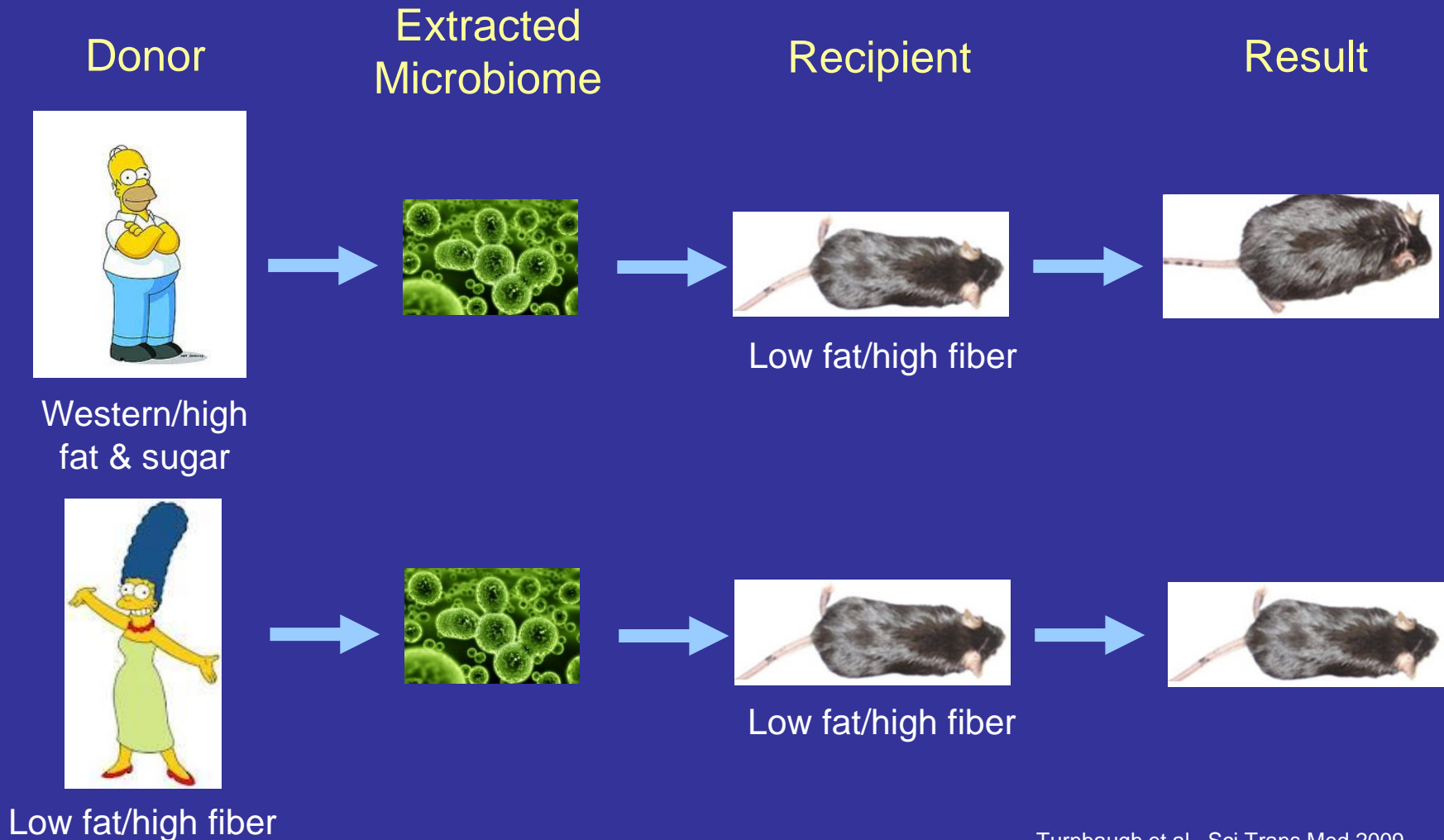
Change in short-chain fatty acids

Change in left-over calories

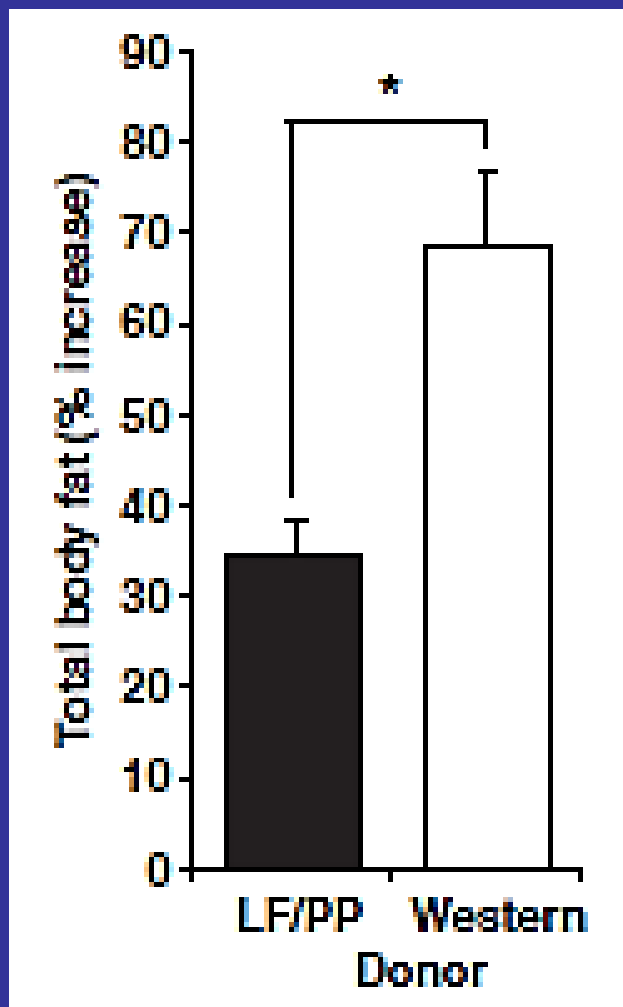
Change in body fat



# Determining cause and effect



# Human obese microbiome → mouse obesity



- Western-fed, obese human donor vs. low fat/high-fiber fed lean human donor
- Post-transplant low fat/high fiber diet in recipient mouse for 2 weeks
- Difference of 34%
- Microbiome profile and phenotype transmitted to next generation

# Metabolic syndrome

- Increased waist circumference
- High triglycerides
- Low HDL
- High blood pressure
- High blood sugar

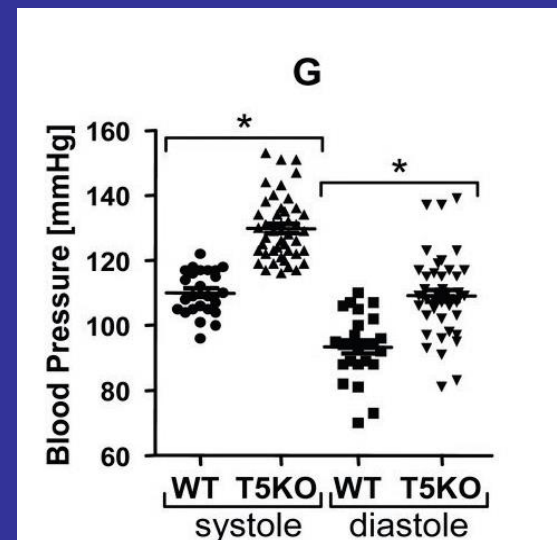
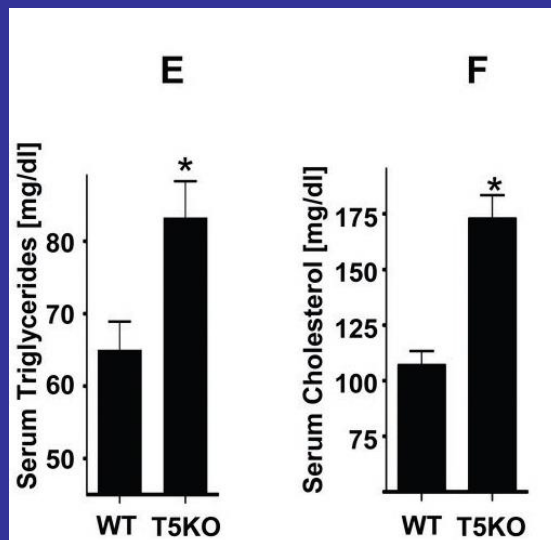
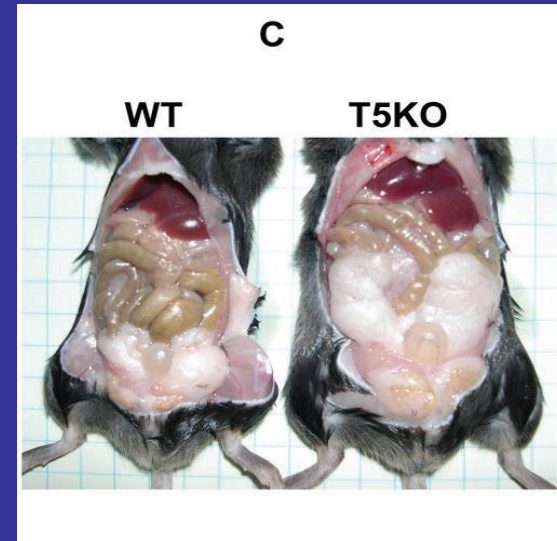
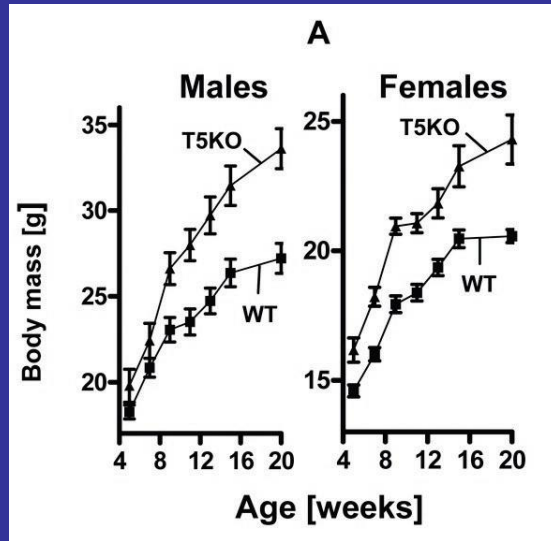
US Age-adjusted prevalence of 34%

# Metabolic syndrome, host immunity and the gut microbiome

- TLR5 – transmembrane protein highly expressed in intestinal mucosa that recognizes bacteria flagella
- Knock-outs: body mass 15% higher than WT littermates
- 60% - elevated pro-inflammatory gene expression

Knock-outs = good metabolic syndrome model

# Metabolic result of TLR5 knock-out





# Potential mechanism = Microbiome



- Treated with Antibiotics
- Lowered bacterial load by 90%
- Correction of metabolic syndrome
  - Lowered glucose levels
  - Lowered food intake
  - Reduced fat pads

# Microbiome mechanisms evidence



## Microbiome transfer:

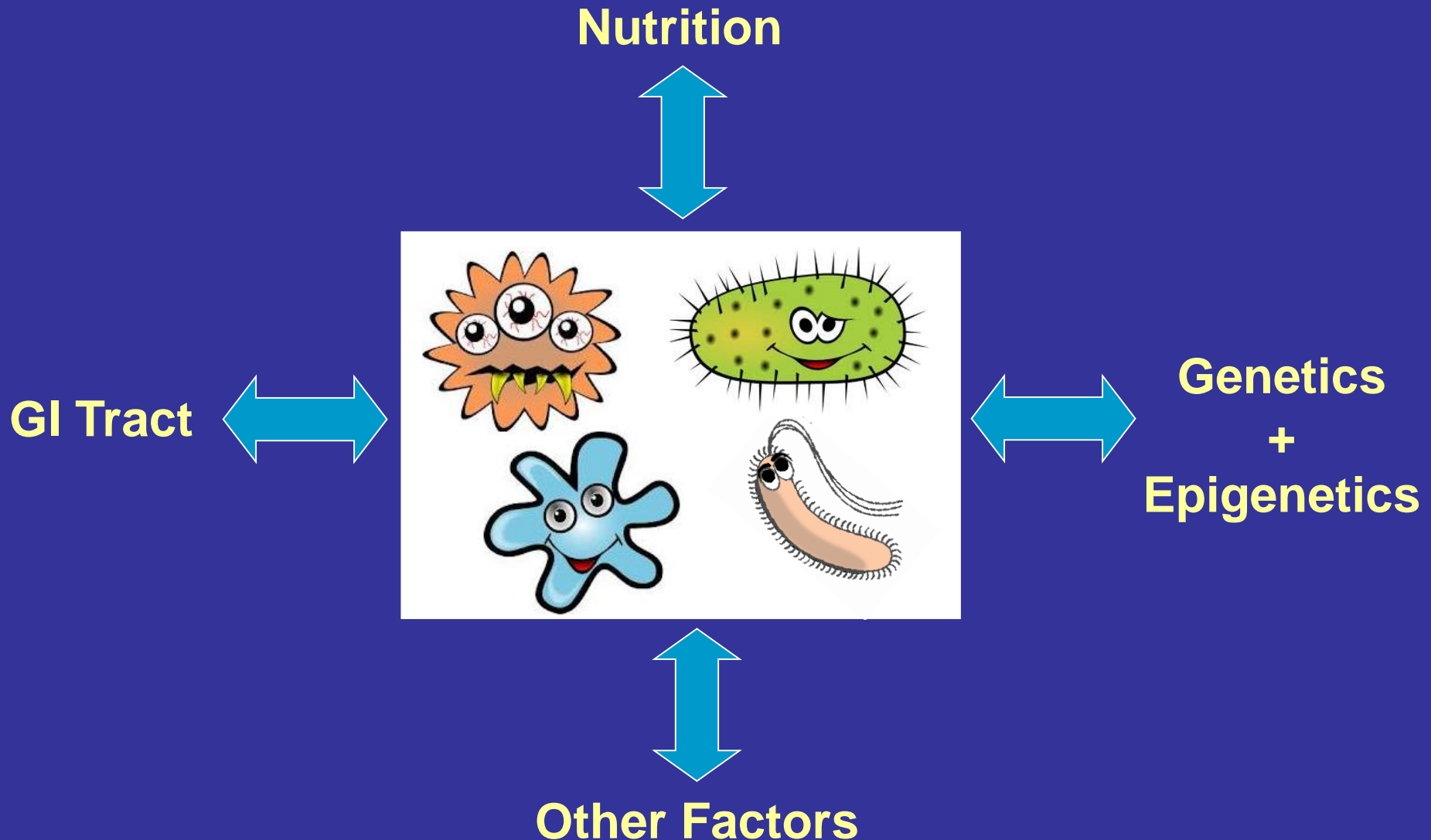
- Increased food intake
- Increased weight gain
- Increased fat pads
- Increased blood glucose
- Increased inflammatory cytokines



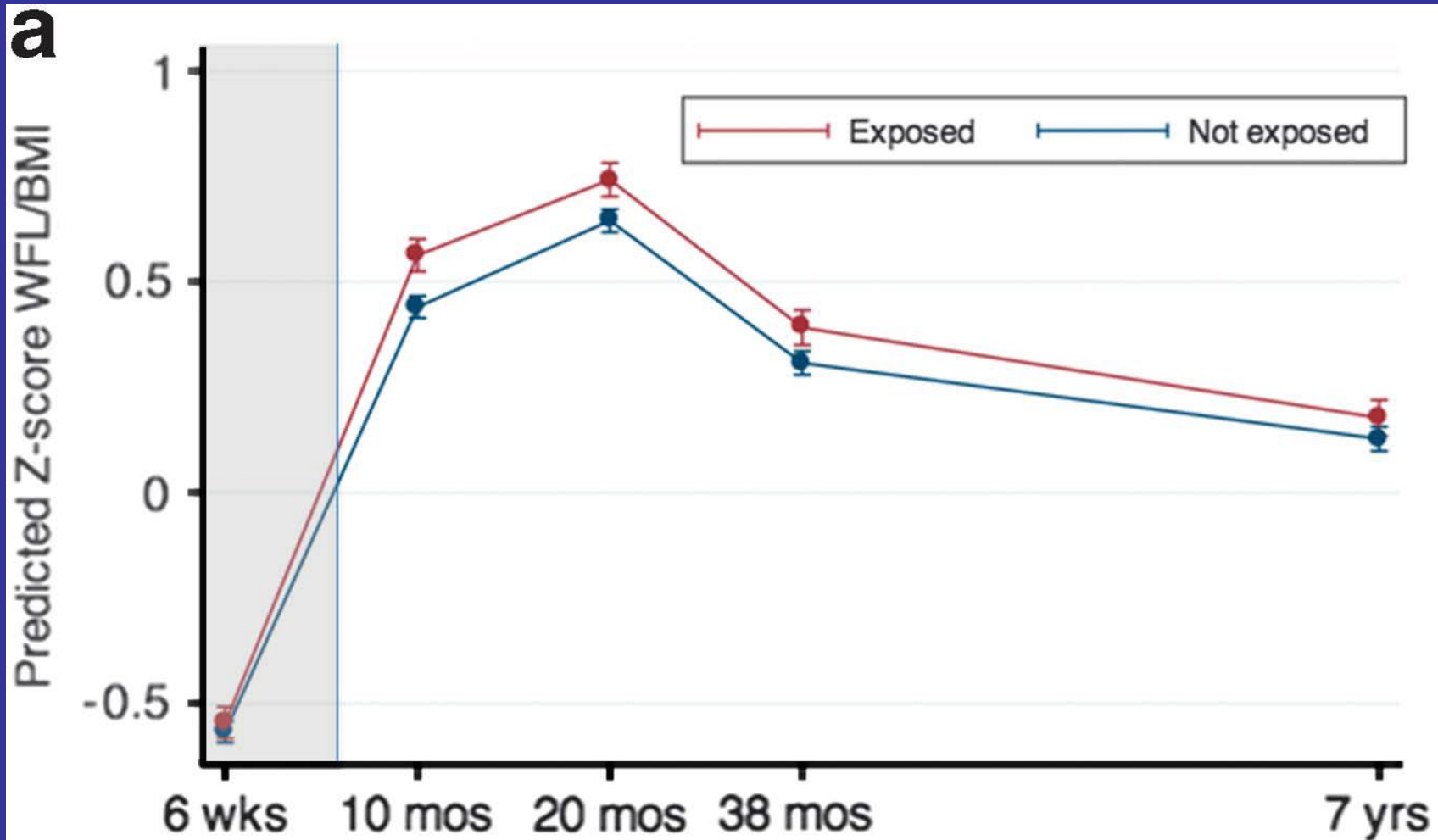
# Diet, the gut microbiome and health: Conclusions

- The microbiota of human populations with different dietary patterns have adapted to their nutritional environments.
- Diet plays an important role in regulating the composition of the gut microbiome.
- Microbiome composition affects host characteristics, such as body weight.
- Genetics also plays a role in microbiome health effects.
- Microbiome transfer experiments demonstrate that genetically associated phenotypes, such as obesity and metabolic syndrome, are transmissible through the gut microbiome alone.

# Antibiotics, Probiotics and the gut microbiome



# Early gut microbiome disruption



# Early gut microbiome disruption



Microbiome recovers post Abx  
BUT  
Metabolic phenotype persists

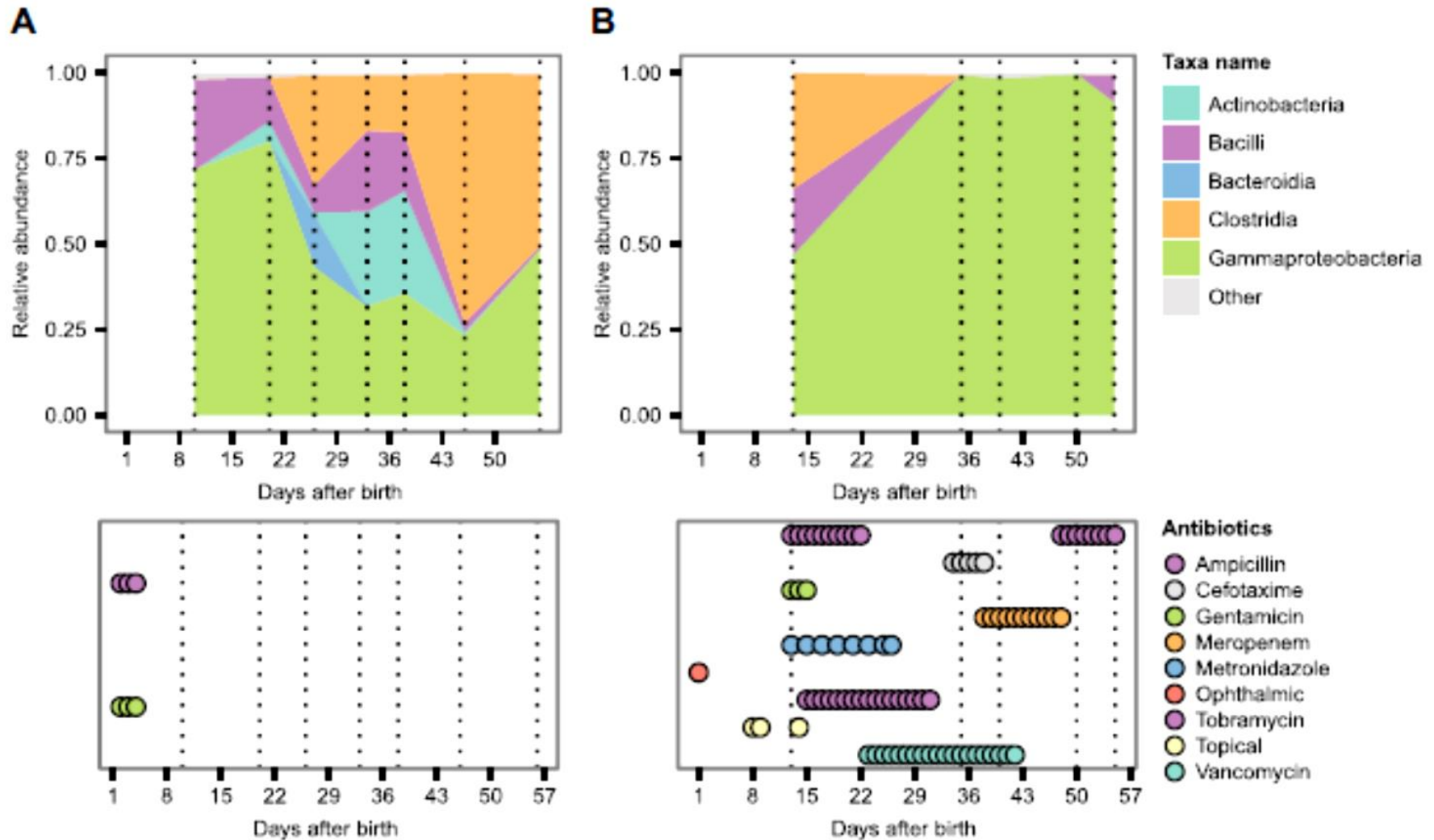
# Antibiotic studies in mice



## Conclusions:

- Abx selection of microbiome maintained
- Microbiome change is causative
- Early life Abx exposure = metabolic changes
- Specific types of bacteria may be biomarkers

# Early life antibiotic exposures - observational





# Early life antibiotic exposures - observational



## Allergies<sup>1</sup>

Milk

(OR 1.78)

Non-milk food

(OR 1.65)

Other

(OR 3.07)

## Asthma

Maternal<sup>2</sup>

(OR 1.21-1.72)

Child – respiratory<sup>3</sup>

(OR 4.12)

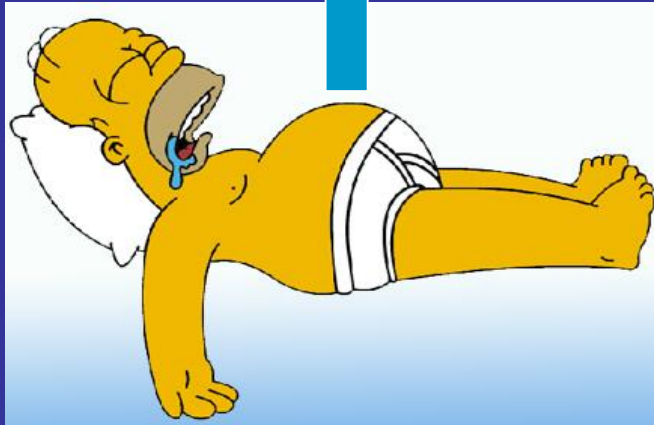
1. Hirsch et al.; Clin Exp Allergy, 2017
2. Chu et al.; PLoS ONE, 2015
3. Ortqvist, et al.; The BMJ, 2014

# Clostridium difficile

- A spore-forming, toxin-producing gut bacterium
- People can carry C. dif with no infection
- Infection produces fever, diarrhea, nausea
- Abx exposure prior to infection is key risk
- Easily transmitted person-to-person
- Antibiotic resistance is increasing
- Difficult to treat, especially Abx resistant strains
- Complications are serious but mortality is fairly low



# Microbiome as Therapy

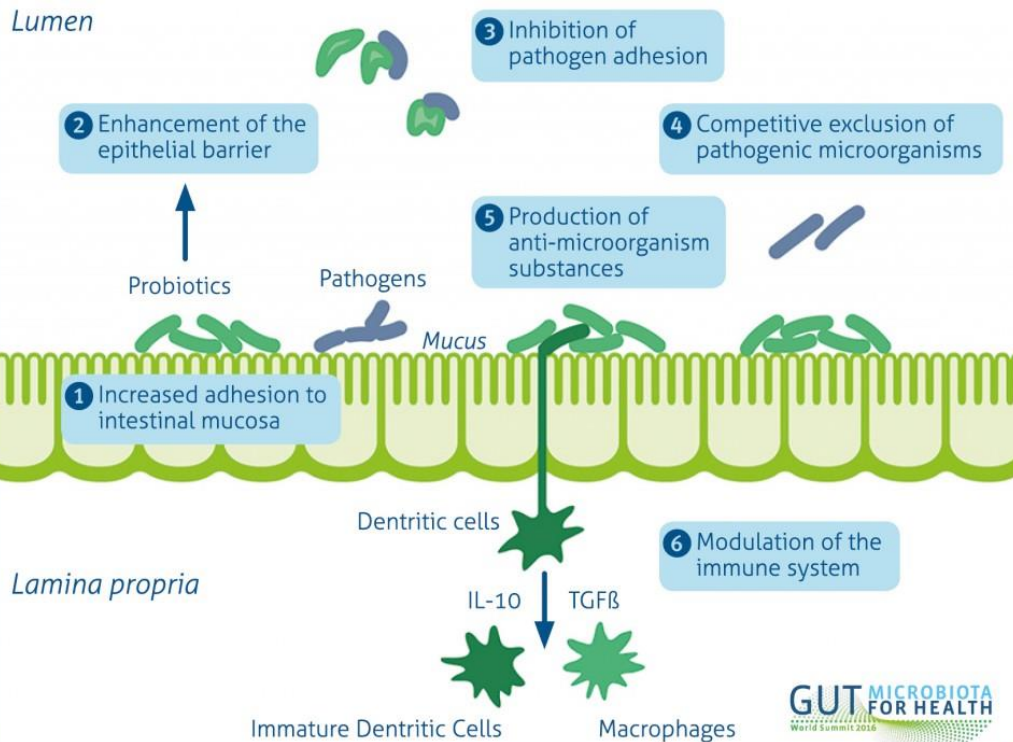


Clostridium difficile



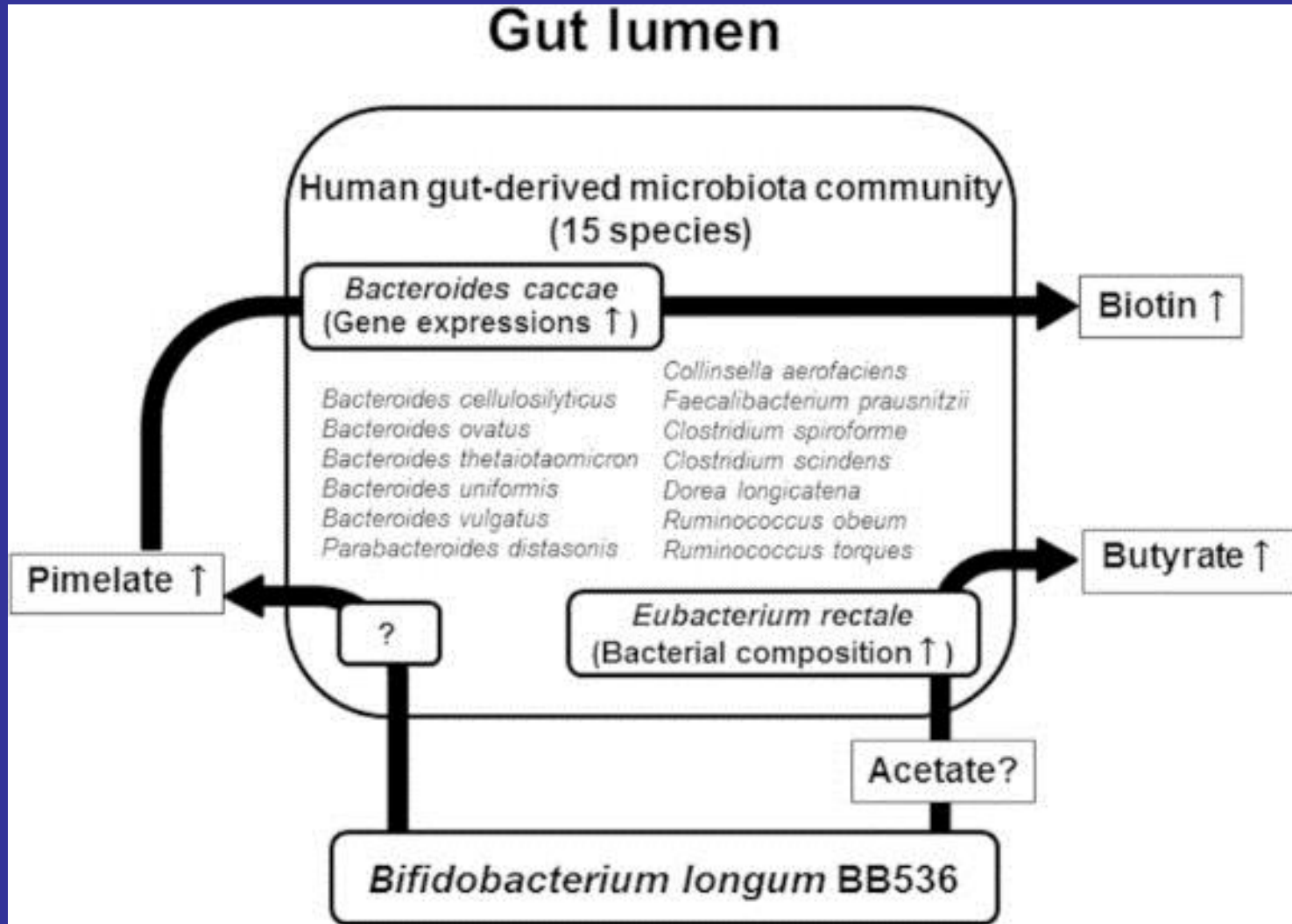
# The Evidence for Probiotics

## Major mechanisms of action of probiotics



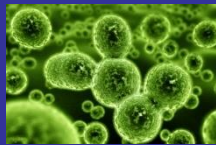
- Probiotic development – gold standard is double-blind, placebo-controlled, randomized trials (RCTs)
- RCTs begun but need larger trials
- Generalizability versus personalization
- Effective and safe dose is not clear for everyone
- Which bacteria for which problems is complex

# One proposed probiotic/micronutrient pathway



# Identifying underlying probiotic mechanisms

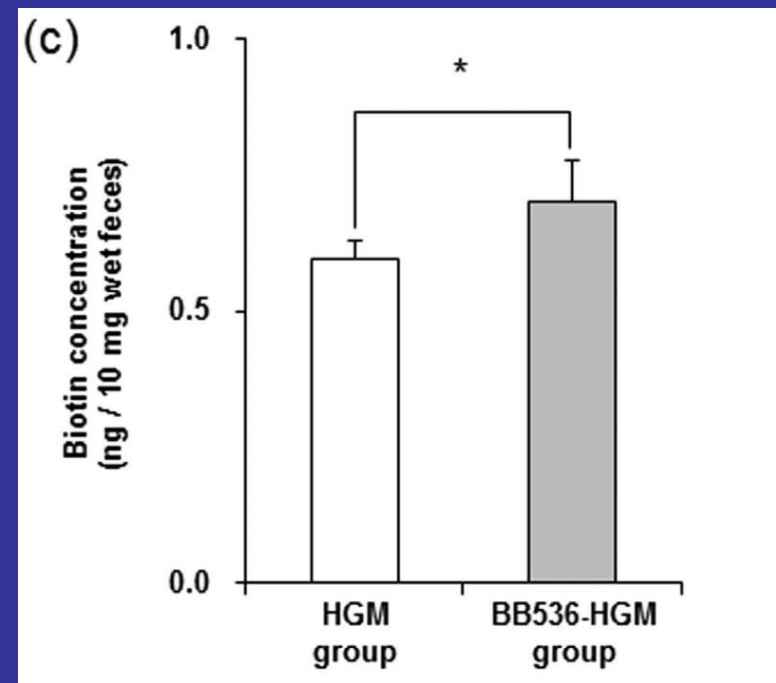
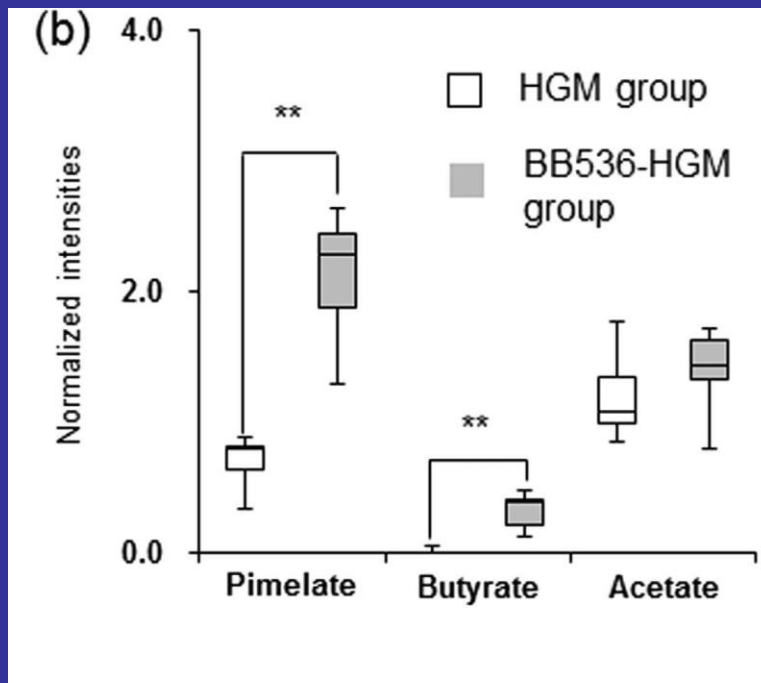
Human  
Microbiome



Recipient

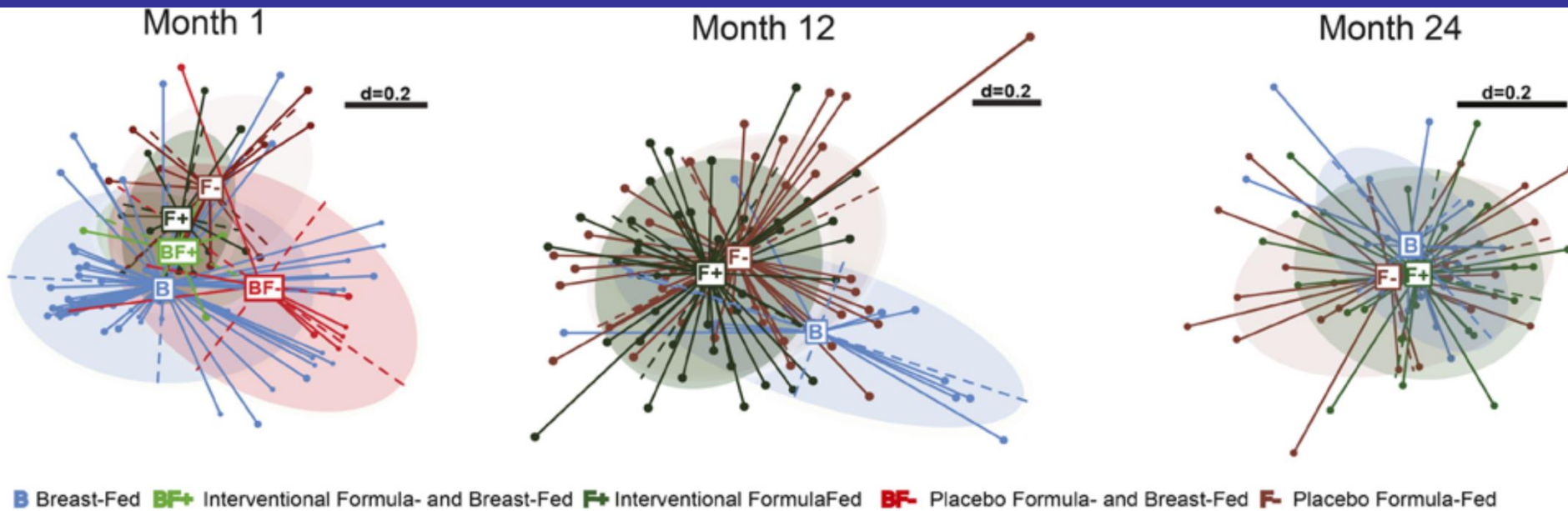


Bifidobacterium  
longum BB536





# RCT: Impact of early-life intervention with bifidobacteria on the healthy infant fecal microbiota and metabolome



## Results:

- 1 month: Microbiome differences segregated by feeding group
- 12 months: Formula fed converged, breast-fed segregated
- 24 months: No discernable differences between groups

Bottom line: Individual differences larger than feeding differences

# Human Probiotic Studies

Study Design	Topic	# Subj.	Results
RCT, Placebo <sup>1</sup>	Gestational Diabetes Mellitus	60	↓ Oxidative Stress ↓ Inflammation Markers = Pregnancy Outcomes
RCT, Placebo <sup>2</sup>	Rheumatoid Arthritis	60	No effect on serum lipids
Double-blind, Placebo, RCT <sup>3</sup>	Alzheimer's	60	↑ Cognitive function ↑ Metabolic status ↓ Markers

1. Badehnoosh et al.; J Matern Fetal Neonatal Med, 2017
2. Vaghef-Mehraba et al.; Health Promotion Perspectives, 2017
3. Akbari et al.; Frontiers in Aging Neuroscience, 2016



# IOC Consensus Statement: Dietary Supplements and the High-Performance Athlete

Probiotics are live micro-organisms that, when administered orally for several weeks, can increase the numbers of beneficial bacteria in the gut. These have been associated with a range of potential benefits to gut health, as well as modulation of immune function.

- Moderate support in athletes with daily dose of  $\sim 10^{10}$  live bacteria
- Cochrane review of 12 studies (n=3720) shows  $\sim 50\%$  decrease in URS incidence and  $\sim 2$  day, shortening of URS; minor side effects.
- More evidence is required supporting efficacy to reduce gastrointestinal distress and infection, for example, in a travelling athlete.



# Antibiotics, probiotics and the gut microbiome: Conclusions

- Antibiotics in gestation and early infancy appear to have long-term effects on microbiome composition and evidence suggests effects on body weight, allergies and asthma.
- Effects of early antibiotic treatment on the microbiome continue into adulthood and are transmissible through the microbiome.
- Antibiotic exposure can reduce the microbiome's ability to fight bad bacteria, resulting in opportunistic infections such as *Clostridium difficile*.
- Fecal transplant is a new and very effective treatment for people with *Clostridium difficile* infections and may help for other conditions.
- Mouse systems are valuable to establish probiotic mechanisms.
- Placebo-controlled randomized clinical trials in humans have begun to document evidence of probiotic benefits but sample sizes need to be larger to understand effects and which bacteria provide the benefit.