gut microbiome, diet, and human health

Thursday, June 14
1pm EST / 10am PST

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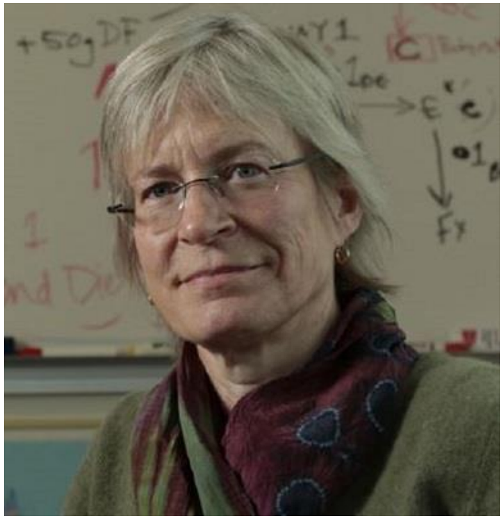
webinar speaker & disclosures

Johanna W. Lampe, PhD, RD



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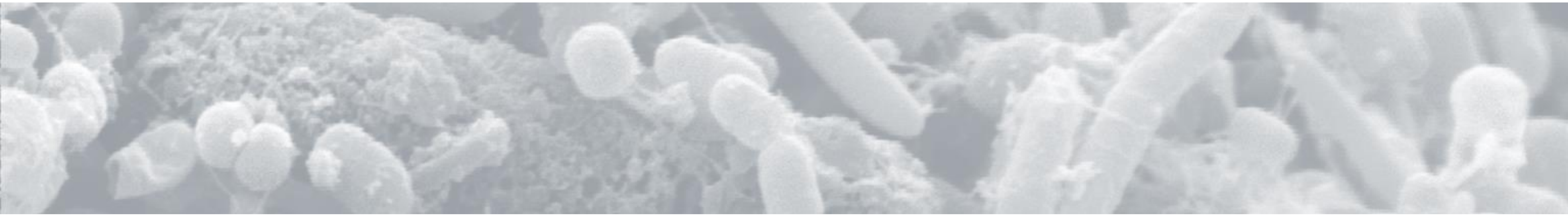
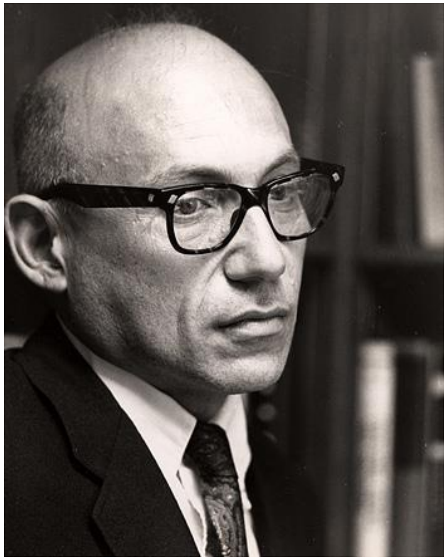
- affiliation/financial interests (past 12 months):
  - grants/research support: NIH
  - Scientific Advisory Board/Consultant: US Highbush Blueberry Council Health Research Committee

outline

- introduction to the gut microbiome
- diet’s effect on the gut microbiome
- gut microbiome’s effect on diet
- pro-, pre- and synbiotics
- potential contributions of fermented foods
- impact of the gut microbiome on human health
- consumer takeaways



what is the microbiome?



> “The ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space.”

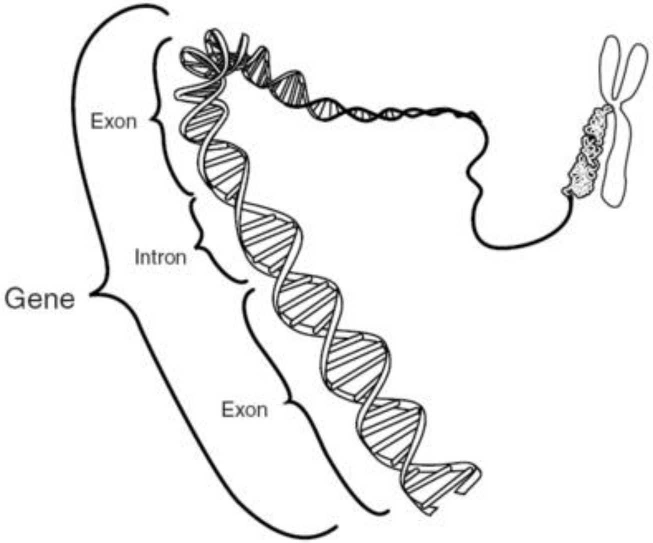
Joshua Lederberg
1925-2008

Lederberg and McCray, *The Scientist*, 2001

microbiome vs microbiota?

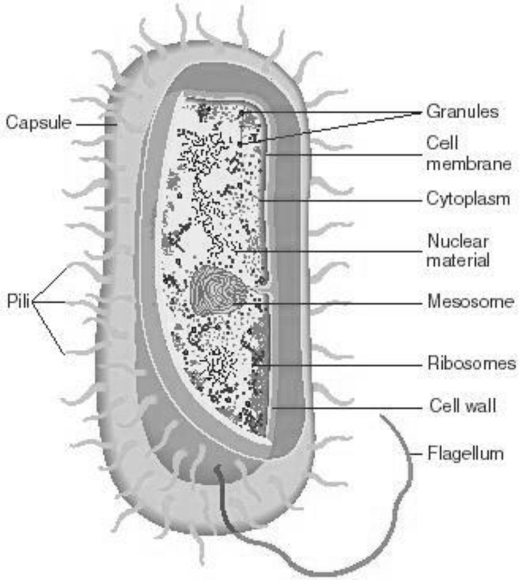
microbiome: all the genes in the microbial community

collective genetic material



microbiota: all community members, including bacteria, archaea, and eukarya

collective microbes



the gut microbiome: who is there?

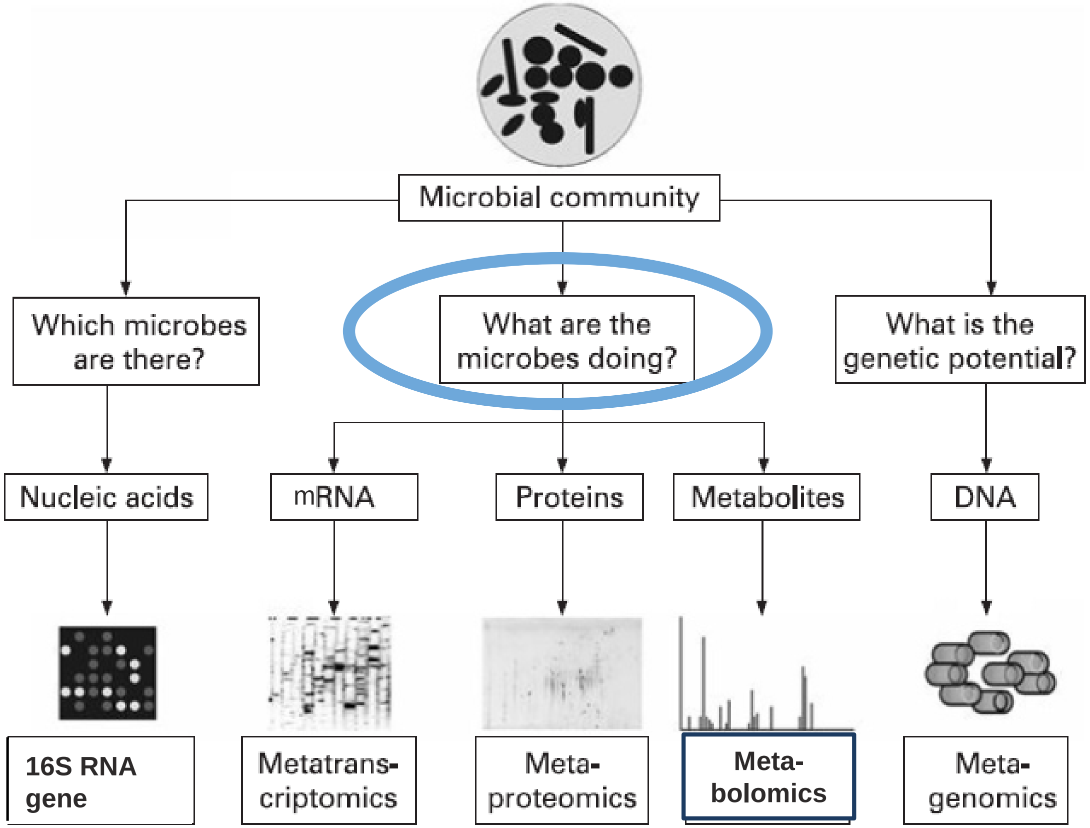
bacteria

viruses

protozoa

fungi

characterizing the gut microbial community: who is there and what are they doing?


Microbial community

Which microbes are there?

What are the microbes doing?

What is the genetic potential?

Nucleic acids

mRNA

Proteins

Metabolites

DNA

16S RNA gene

Metatrans-criptomics

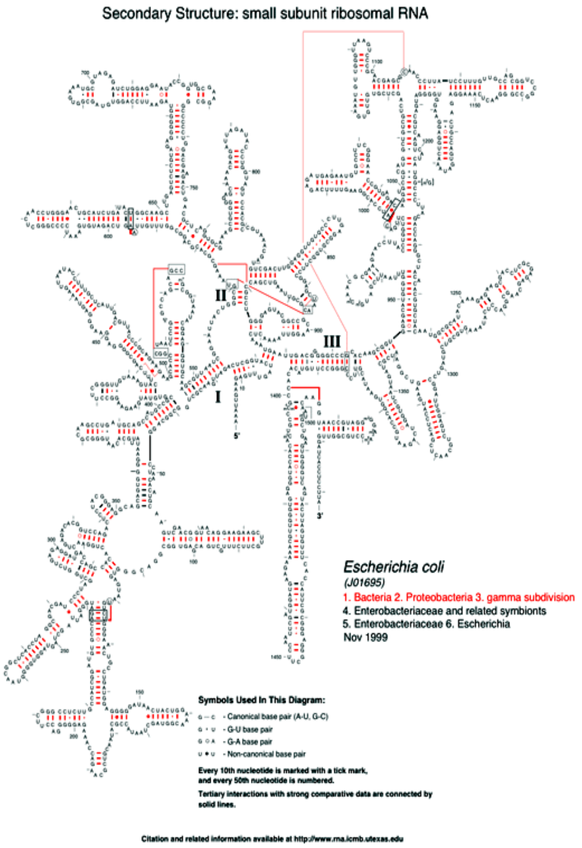
Meta-proteomics

Meta-bolomics

Meta-genomics

Zoetendal et al., *Gut*, 2008

characterizing the gut microbiome with 16s rRNA genes: who is there?



Secondary Structure: small subunit ribosomal RNA

Escherichia coli
(J01695)
1. Bacteria 2. Proteobacteria 3. gamma subdivision
4. Enterobacteriaceae and related symbionts
5. Enterobacteriaceae 6. Escherichia
Nov 1999

Symbols Used In This Diagram:
G — C - Canonical base pair (A-U, G-C)
G • U - G-U base pair
G ○ A - G-A base pair
U ● U - Non-canonical base pair

Every 10th nucleotide is marked with a tick mark, and every 50th nucleotide is numbered.

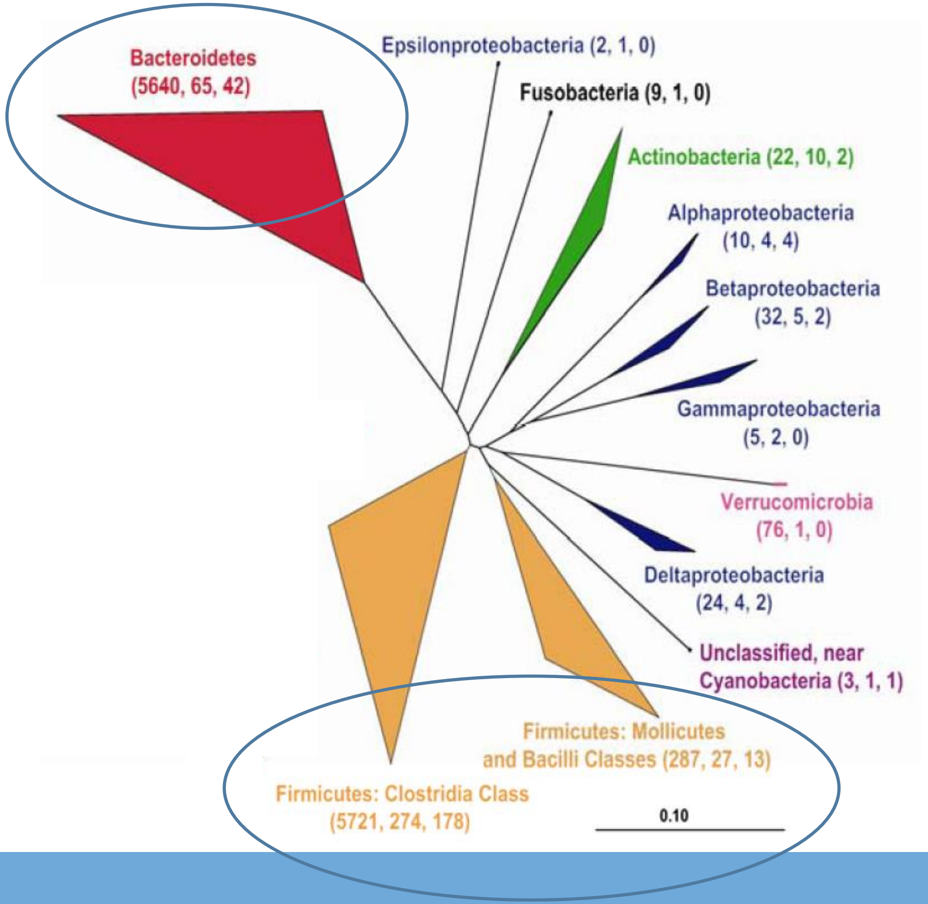
Tertiary interactions with strong comparative data are connected by solid lines.

Citation and related information available at http://www.rna.icmb.utexas.edu

- component of small subunit of prokaryotic ribosomes
- highly conserved in different species of bacteria and archaea
- no horizontal gene transfer
- hyper-variable regions provide species-specific signature sequences for bacterial identification.

bacterial diversity in the adult human gut

*kingdom • **phylum** • class • order • family • genus • species • strain*

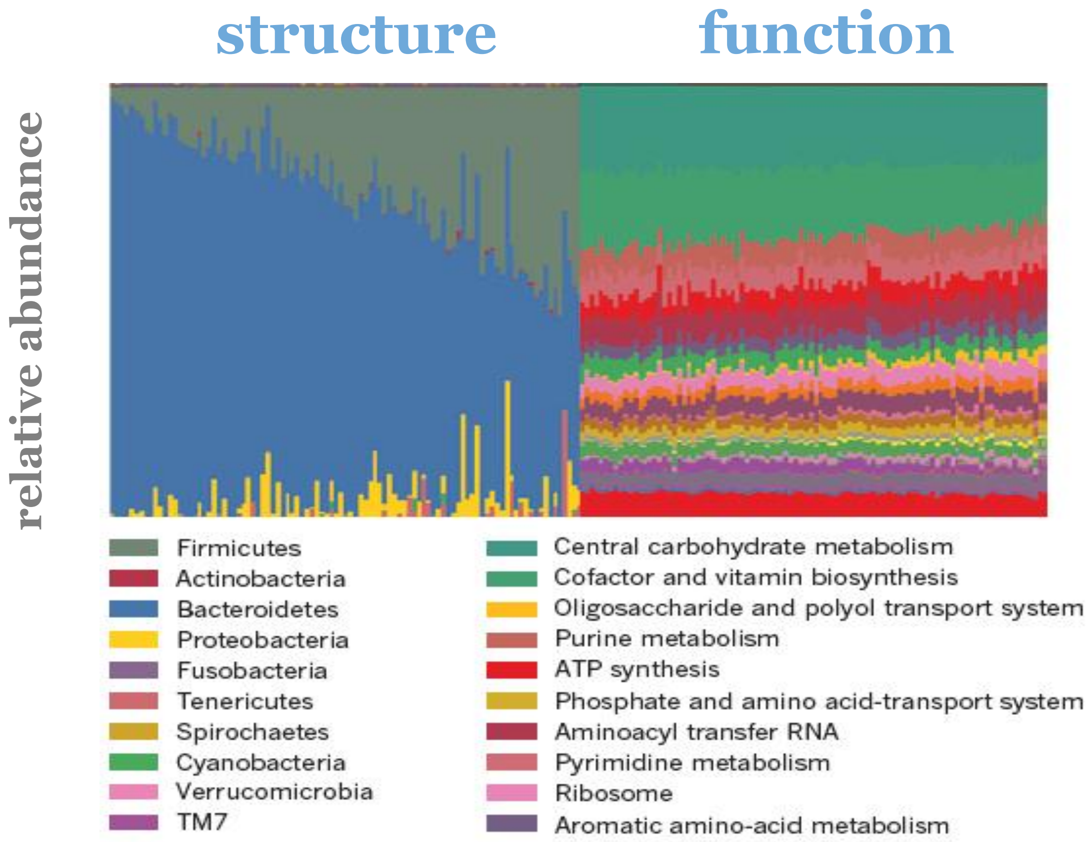


At least 10 different phyla of bacteria found in the human gut

- 5 phyla represent majority of bacteria
- bacteroidetes and firmicutes dominate.
- 100s of species

Bäckhed et al. *Science* 2004
Marchesi et al, Gut, 2016

functional redundancy: structural (taxonomic) vs functional variation in the gut microbiome



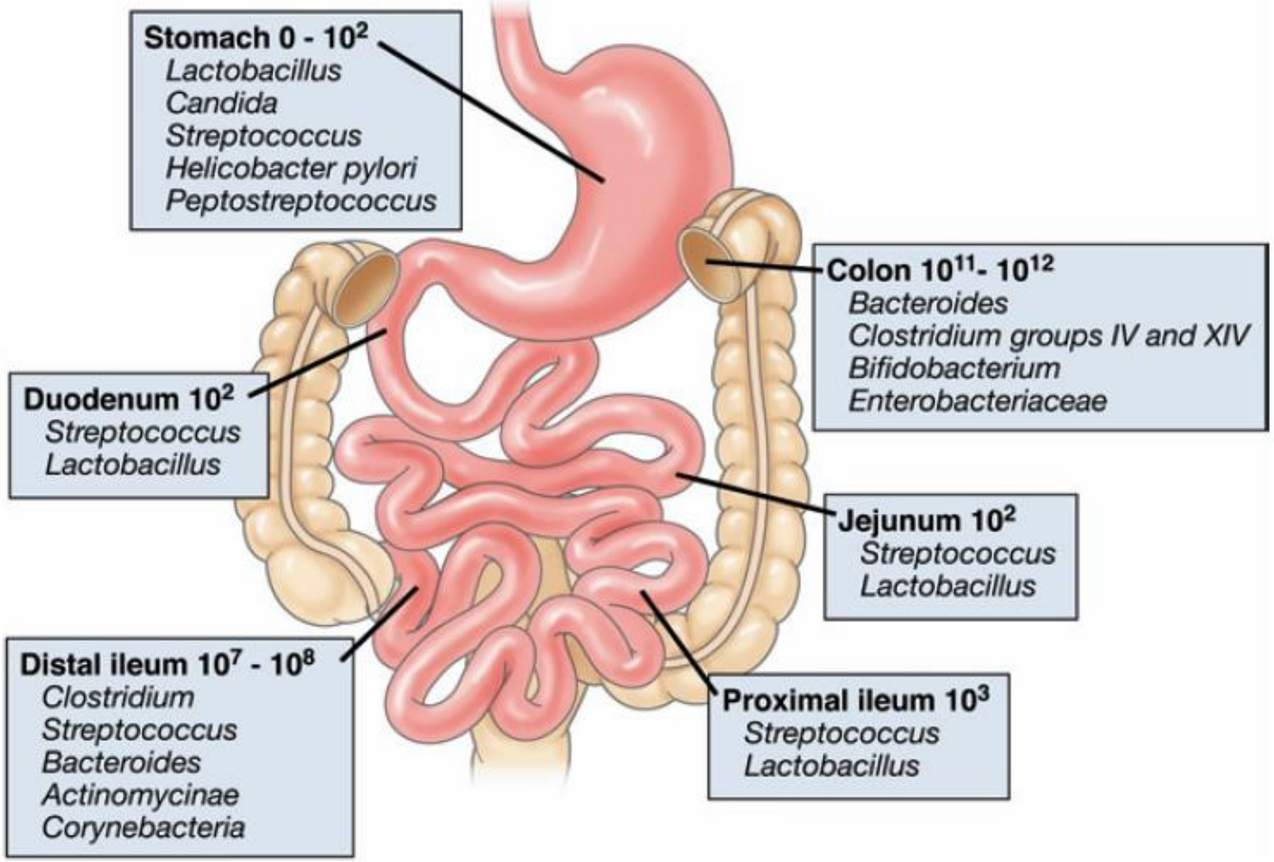
Human Microbiome Project
Consortium. *Nature* 486:207, 2012

diet's effect on the gut microbiome

gut microbiome's effect on diet

the human gut microbiome

composition and luminal concentrations of microbial groups vary along the GI tract.



Stomach 0 - 10^2
Lactobacillus
Candida
Streptococcus
Helicobacter pylori
Peptostreptococcus

Colon 10^{11}- 10^{12}
Bacteroides
Clostridium groups IV and XIV
Bifidobacterium
Enterobacteriaceae

Duodenum 10^2
Streptococcus
Lactobacillus

Jejunum 10^2
Streptococcus
Lactobacillus

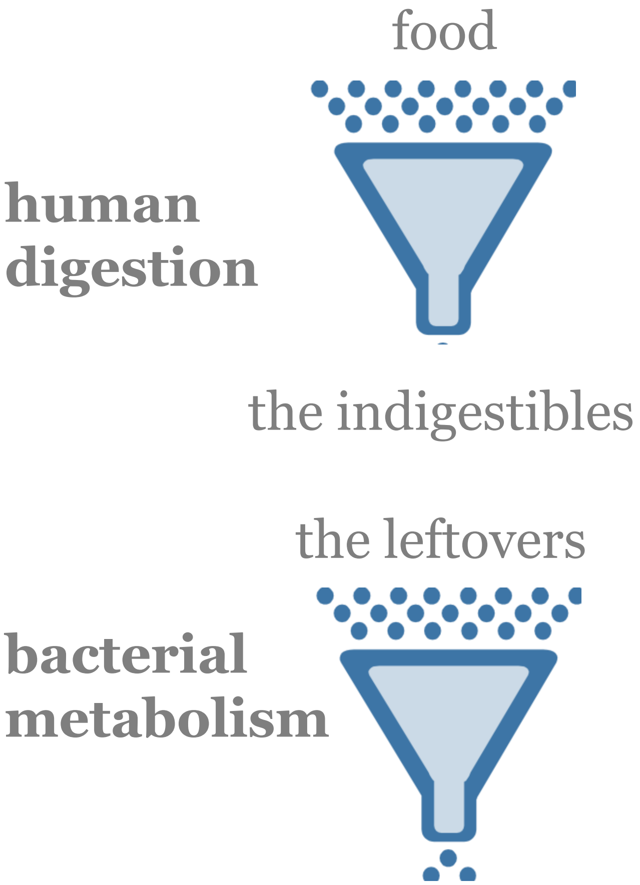
Distal ileum 10^7 - 10^8
Clostridium
Streptococcus
Bacteroides
Actinomycinae
Corynebacteria

Proximal ileum 10^3
Streptococcus
Lactobacillus

Sartor RB, *Gastroenterol*, 134:577,2008

gut microbial metabolism

- obtain energy and nutrients to live and reproduce
- microbiome: >100 times as many genes as human genome
- carry out reactions that human gut enzymes cannot:
  - fermentation
  - denitrification
  - sulfate reduction
  - aromatic fission
  - hydrolysis/deconjugation



food

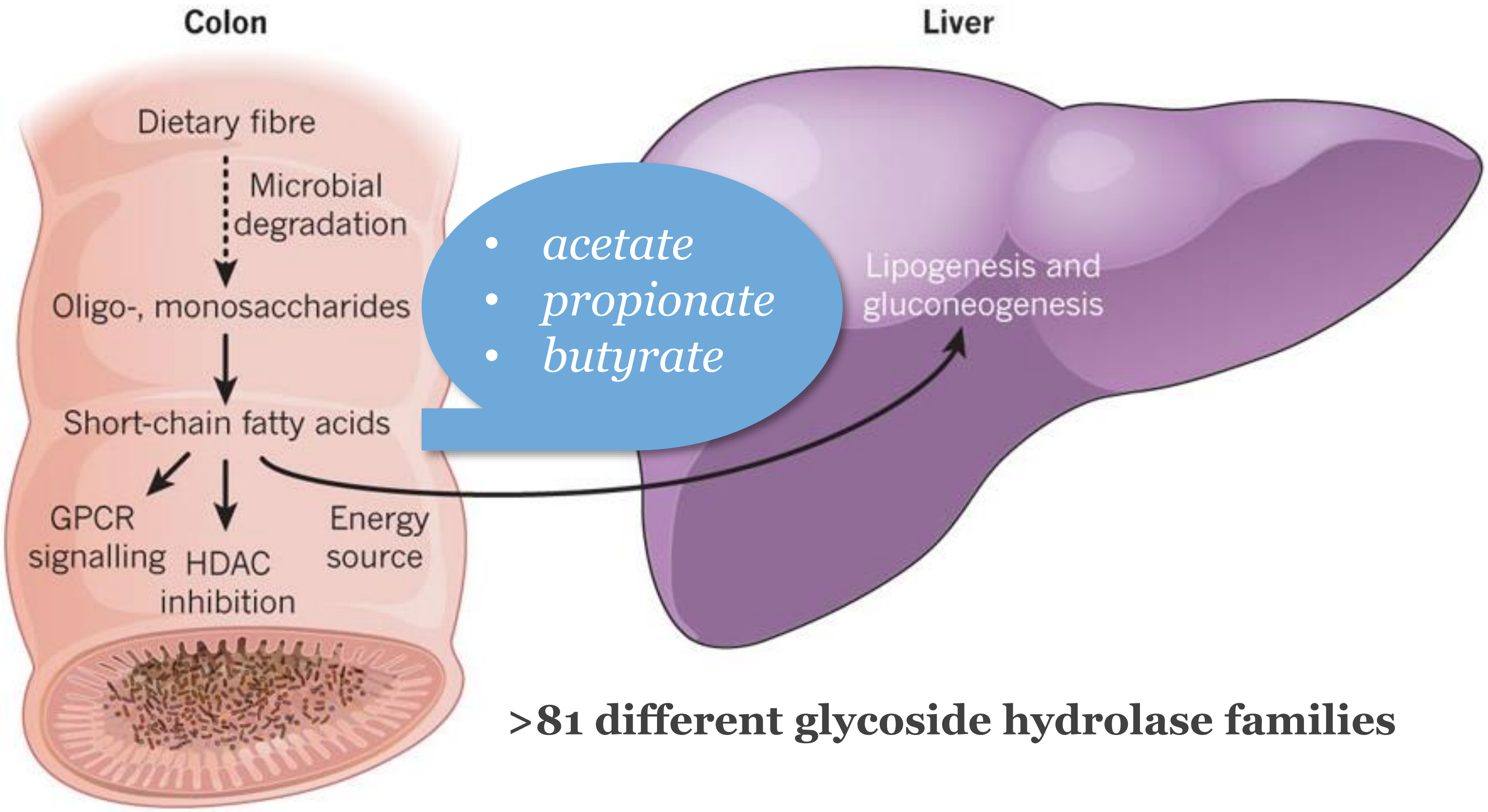
human digestion

the indigestibles

the leftovers

bacterial metabolism

microbial fermentation of carbohydrates: sugars, dietary fiber and resistant starch

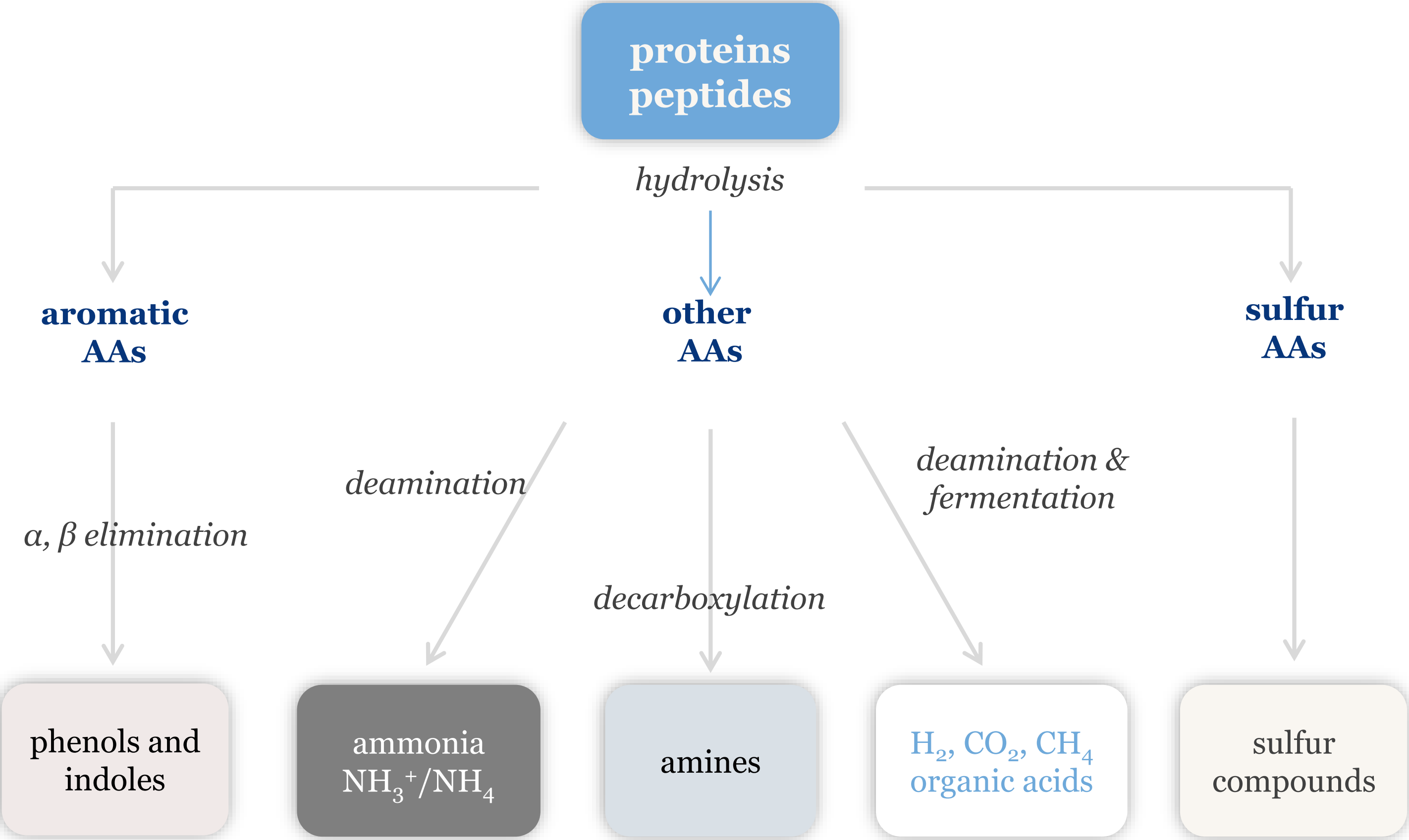


- *acetate*
- *propionate*
- *butyrate*

>81 different glycoside hydrolase families

Gill et al, *Science*, 2006
Tremaroli & Bäckhed, *Nature*, 2012

microbial metabolism of proteins & amino acids



proteins peptides

hydrolysis

aromatic AAs

other AAs

sulfur AAs

α, β elimination

deamination

decarboxylation

deamination & fermentation

phenols and indoles

ammonia NH_3^+/NH_4

amines

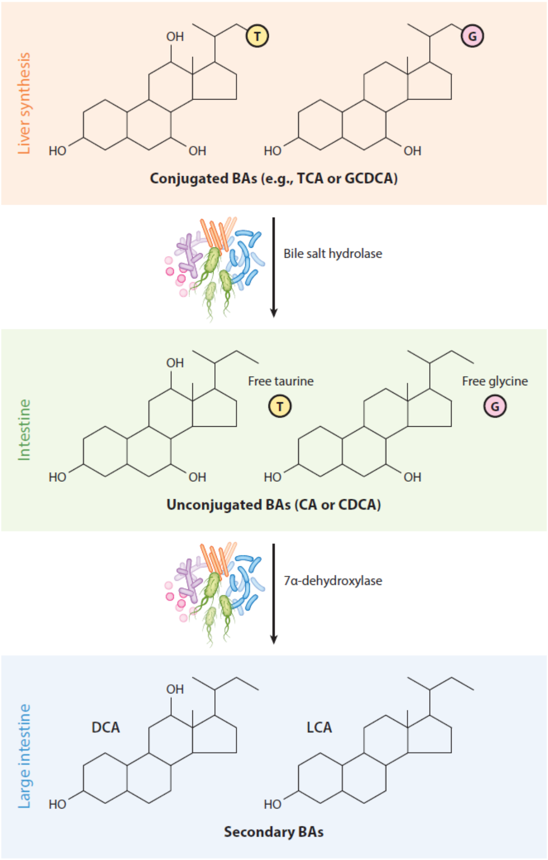
H_2, CO_2, CH_4 organic acids

sulfur compounds

Adapted from Nyangale et al. *J Proteome Res,* 2012

microbial metabolism of dietary fats

- metabolism of lipids
- bile acid metabolism
  - primary to secondary bile acids
  - altered microbial community
  - changes in signaling to liver



Joyce and Gahan, *Ann Rev Food Sci Technol*, 7:313, 2016

diet's effect on the gut microbiome

evidence from:

observational studies

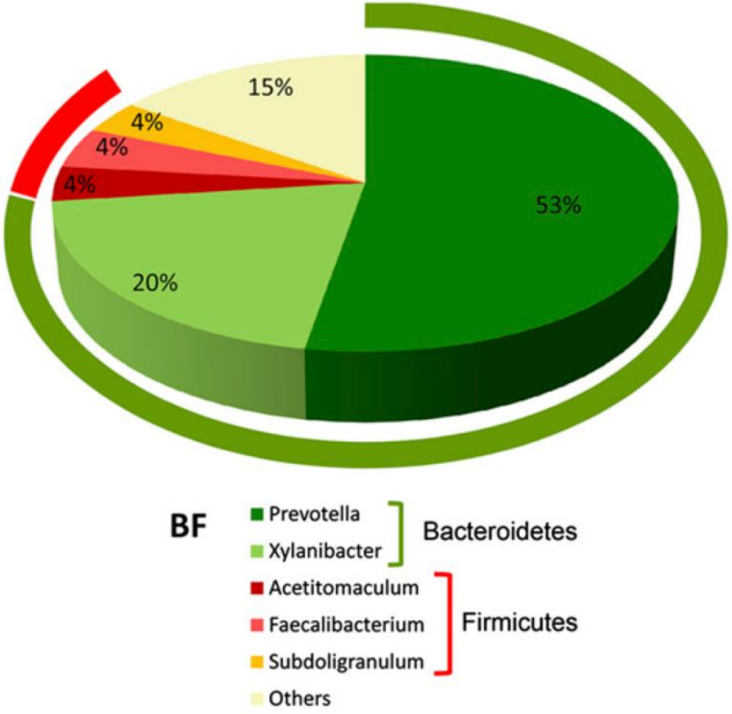
- globally distinct populations
- long-term food pattern consumption

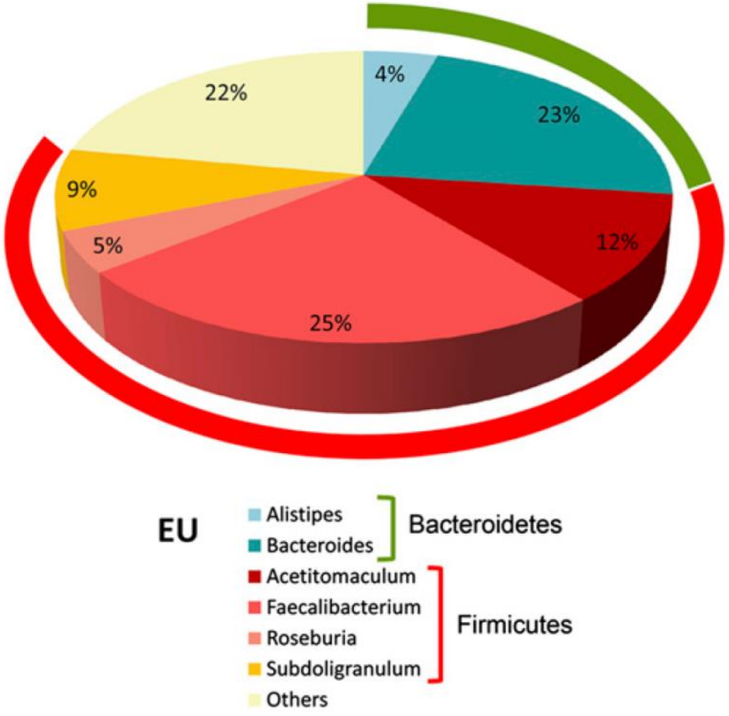
short-term dietary interventions

- low- vs high-fiber diets
- animal vs plant food sources
- macronutrient ratios

Reviewed in Arora & Bäckhed. *J Int Med* 280:339, 2016

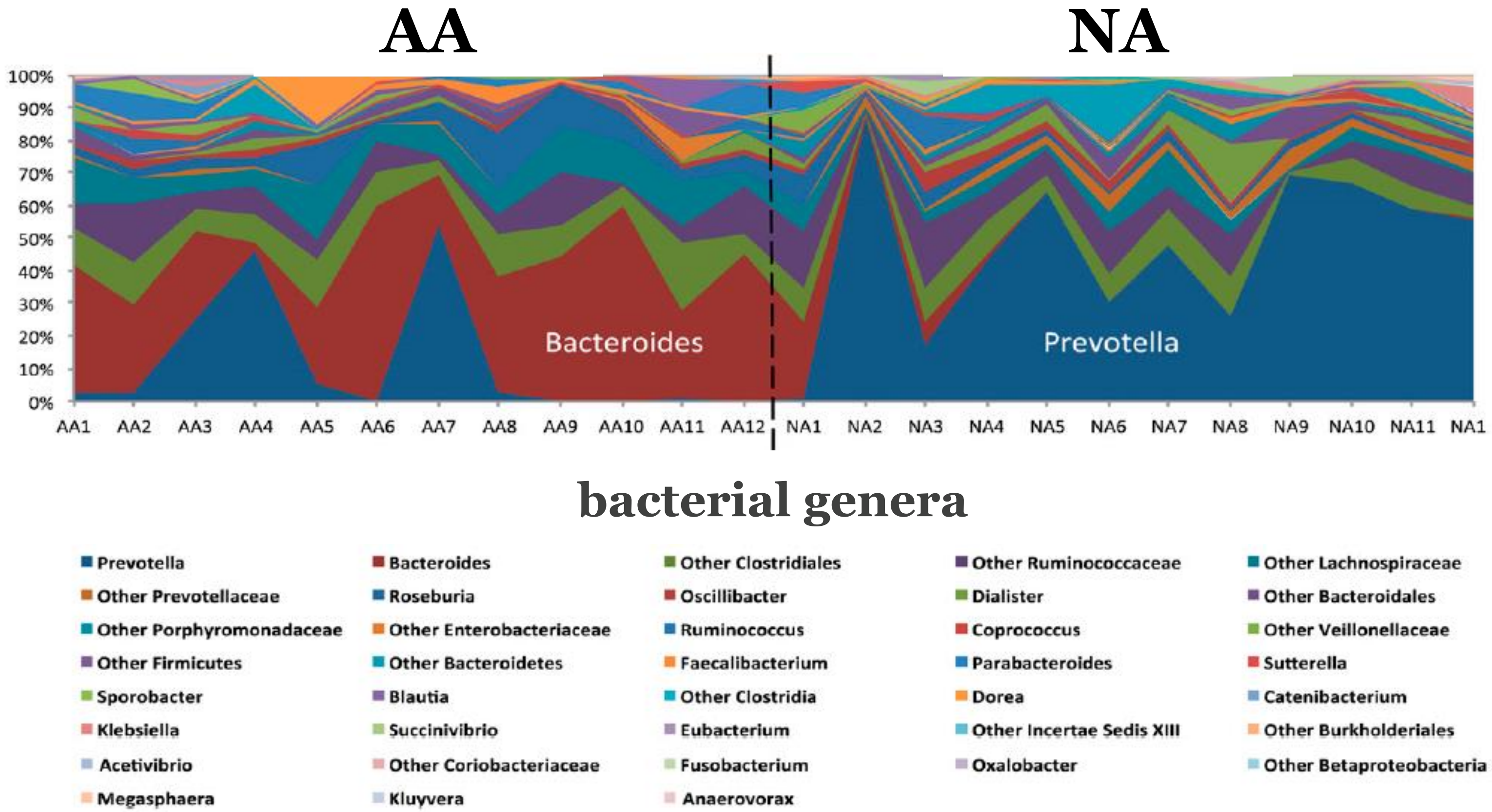
global population differences:
children in rural Africa (BF) vs urban Europe (EU)





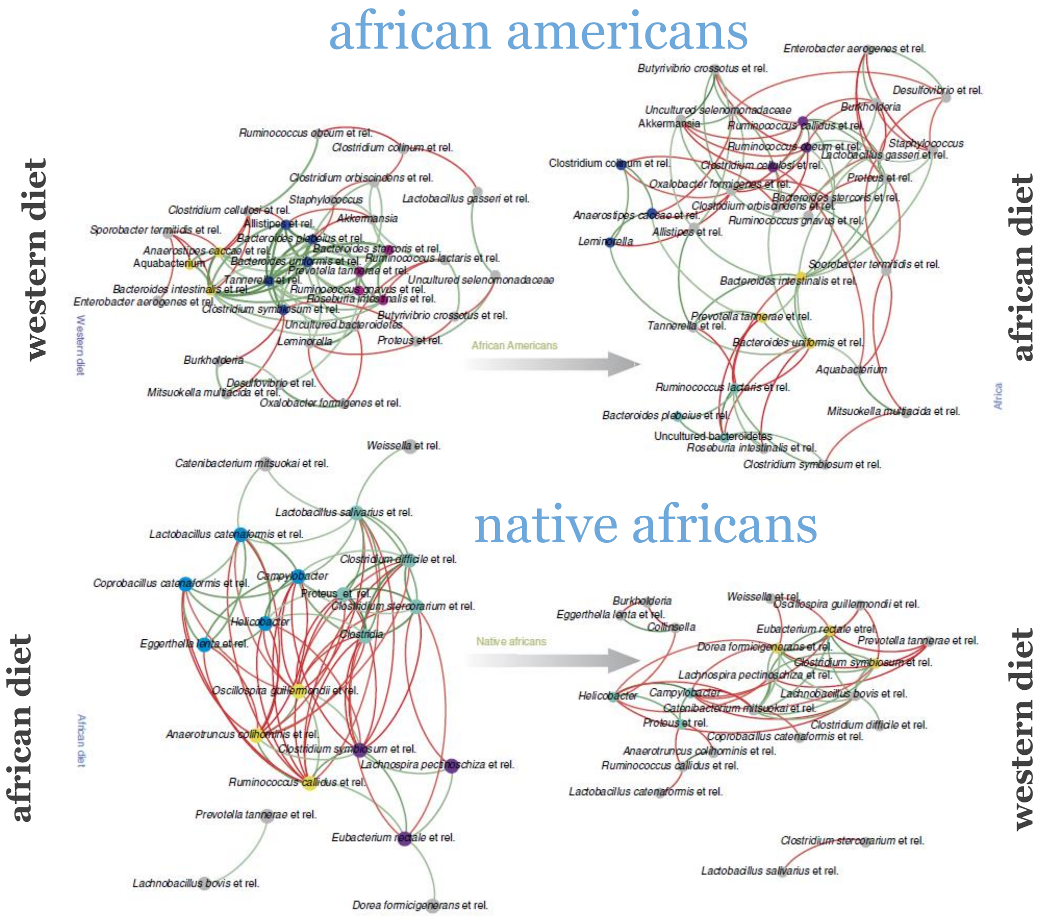
De Filippo et al *PNAS* 107:14691, 2010

global population differences: african-americans in US vs native south africans



Ou et al, *Am J Clin Nutr* 98:111, 2013

microbiota change with diet switch in african americans and native south africans



african americans

western diet

african diet

native africans

african diet

western diet

2-wk feeding study

US AAs fed:
- fiber: 14→55g/d
- fat: 35→16%

native africans fed:
- fiber: 66→12g/d
- fat: 16→52%

O'Keefe et al, *Nat Comm* 6:6342, 2015

diet differences within a population: dietary patterns and gut microbiome

Mediterranean Diet Pyramid

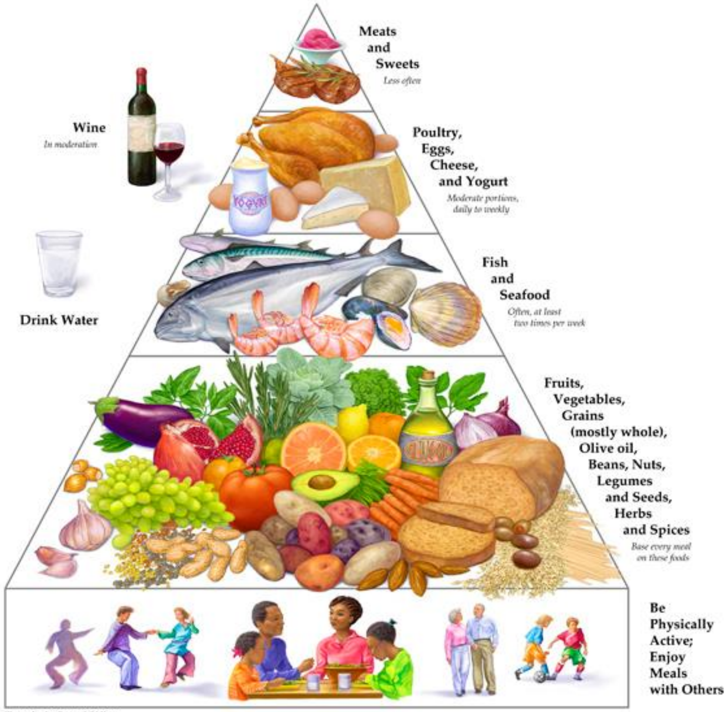


Illustration by George Middleton

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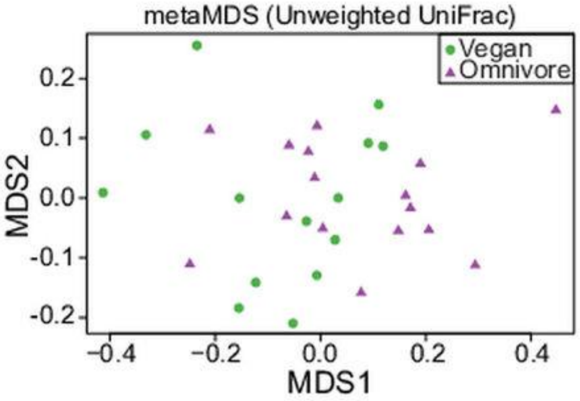
Dietary Patterns:

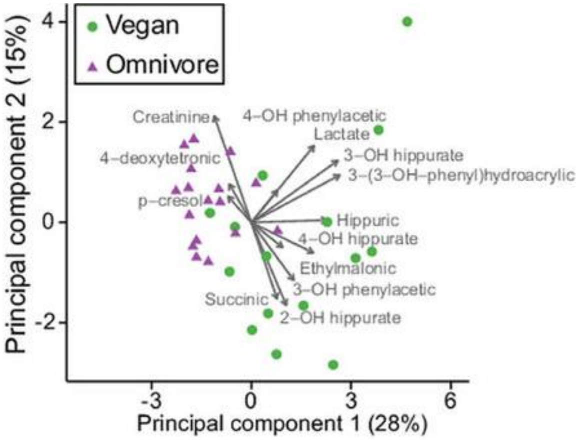
- Healthy Eating Index 2010
- Alternative Healthy Eating Index
- Mediterranean diet
- DASH diet

To date, few studies have examined associations between specific healthy eating patterns and the gut microbiome.

Bowyer et al. *Microbiome* 6:77, 2018

small gut microbial differences in vegans and omnivores in an urban US setting

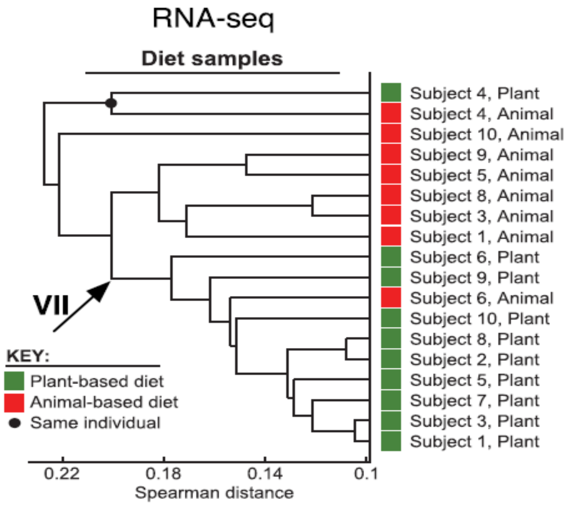


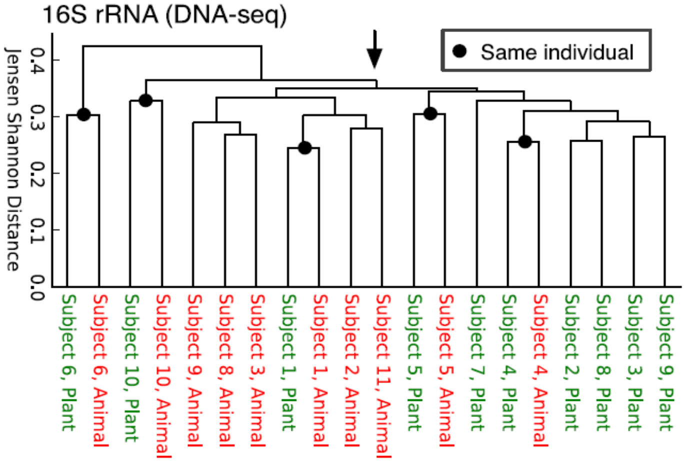


- Sampled 15 vegans and 16 omnivores.
- 16S rRNA sequence data showed:
  - A. Modest difference in composition in unweighted (presence/absence) Unifrac (PERMANOVA, p=0.007).
  - B. No difference in abundance weighted analysis (p=0.15).
- Plasma metabolome differed more substantially.

Wu GD et al. *Gut* 2016;65:63.

short-term feeding of plant- and animal-based diets alters gut microbiota





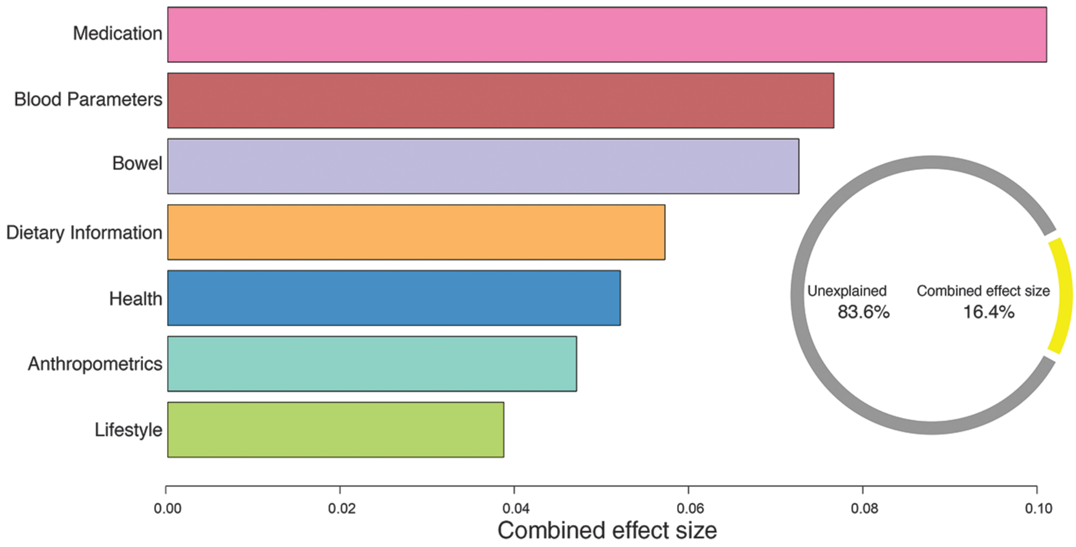
- 10 subjects tracked across 5-d animal- and plant-based diets.

	Baseline	Animal	Plant
Fat, % kcal	32	69	22
Protein, % kcal	16	30	10
Fiber, g/1000 kcal	9	0	26

- Bacterial metabolic gene expression (RNA-seq) tends to cluster by diet.
- Animal-based diet increased bile-tolerant microbes and decreased microbes that ferment fibers.
- Diet doesn't always overcome inter-individual differences in GMC structure (16S rRNA).

David et al, *Nature*, 505:559 2014

what explains gut microbiome composition variation within a homogenous population?



- Belgian Flemish Gut Flora Project (n=1106)
- 69 clinical and questionnaire-based covariates associated with microbiota compositional variation
- Medication use explained largest total variance
- Stool consistency showed largest effect size
- Still only explain ~16% of total variation

Falony et al. *Science* 352:560, 2016

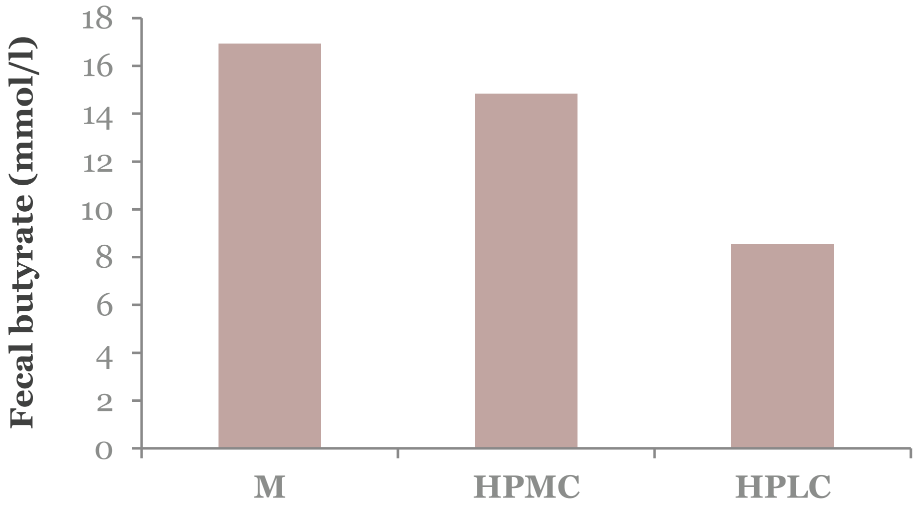
the gut microbiome’s effect on diet

- **alters exposure to nutrients and bioactives**
- **generates new compounds, that:**
  - serve as energy sources
  - regulate metabolism
  - increase or reduce inflammation
  - cause or reduce oxidative stress
  - are genotoxic/carcinogenic

bacteria can produce new compounds from dietary constituents

food component	**bacterial metabolites**
dietary fiber	butyrate and other SCFAs
soy isoflavones	equol, *O*-desmethylangolensin
anthocyanins	hippuric acid & small phenolics
glucosinolates	isothiocyanates

diet-microbial community interaction affects exposure to metabolites



	M	HPMC	HPLC
Protein, %	13	28	29
Fat, %	37	37	66
Carb, %	50	35	5
NSP, g	22	9	13

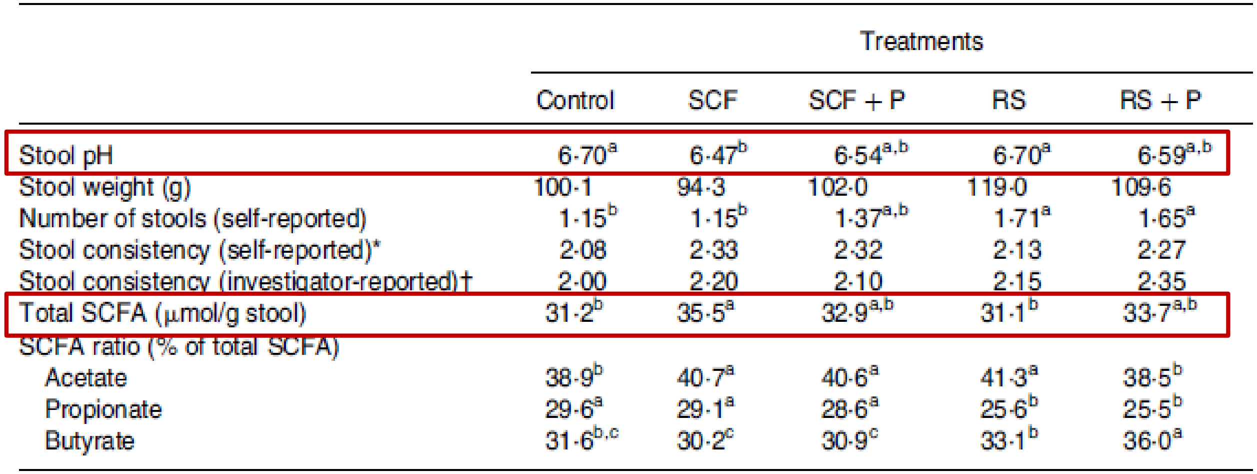
- 17 obese men, randomized cross-over design
- 4 weeks of weight-reduction diets:
  - high-protein; med carb (HPMC)
  - high-protein; low-carb (HPLC)
- HPLC diet decreased butyrate levels

Russell et al., *Am J Clin Nutr* 2011;93:1062.

diet fiber-microbial community interactions affect exposure to short chain fatty acids

- 20 healthy participants, in randomized, double-blind, cross-over design
- five 7-d treatments

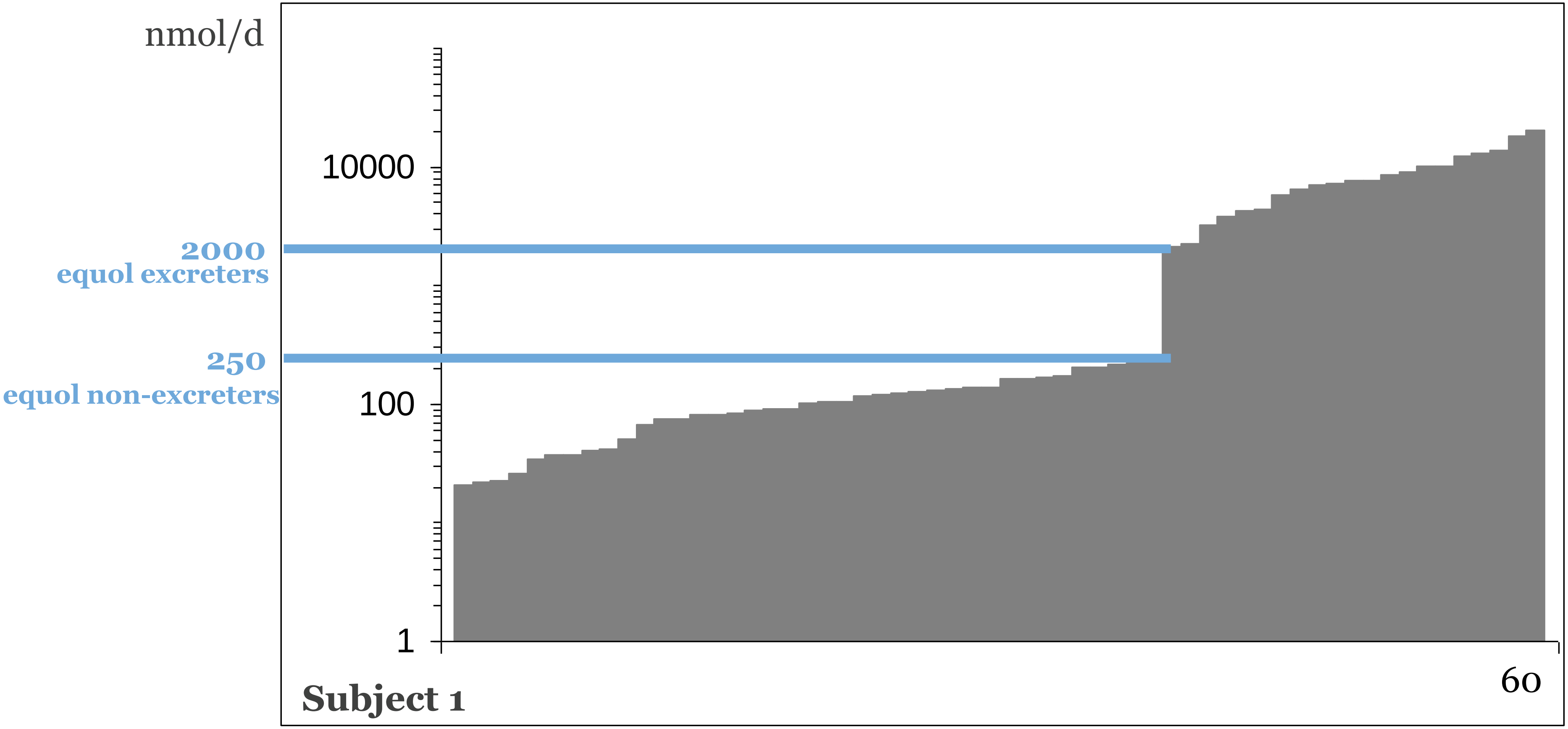
- maltodextrin control
- 20-25 g fiber from
  - soluble maize fiber (SCF)
  - SCF + pullulan (P)
  - resistant starch (RS)
  - RS + P



	Treatments				
	Control	SCF	SCF + P	RS	RS + P
Stool pH	6·70[a]	6·47[b]	6·54[a,b]	6·70[a]	6·59[a,b]
Stool weight (g)	100·1	94·3	102·0	119·0	109·6
Number of stools (self-reported)	1·15[b]	1·15[b]	1·37[a,b]	1·71[a]	1·65[a]
Stool consistency (self-reported)*	2·08	2·33	2·32	2·13	2·27
Stool consistency (investigator-reported)†	2·00	2·20	2·10	2·15	2·35
Total SCFA (μmol/g stool)	31·2[b]	35·5[a]	32·9[a,b]	31·1[b]	33·7[a,b]
SCFA ratio (% of total SCFA)					
Acetate	38·9[b]	40·7[a]	40·6[a]	41·3[a]	38·5[b]
Propionate	29·6[a]	29·1[a]	28·6[a]	25·6[b]	25·5[b]
Butyrate	31·6[b,c]	30·2[c]	30·9[c]	33·1[b]	36·0[a]

AS Klosterbuer et al., *Br J Nutr* 2013;110:1068.

urinary equol excretion with soy challenge: not everyone's gut microbes make equol



Lampe et al., *PSEBM* 217:335-339, 1998

pro-, pre- and synbiotics

defining probiotics, prebiotics and synbiotics

probiotics

- *definition:* live microorganisms which when administered in adequate amounts confer a health benefit on the host
  –WHO

prebiotics

- *definition:* non-digestible carbohydrates which are selectively fermented by the gut microbiota, leading to improvements in health outcomes

- in principle, all dietary fibers that are fermented have prebiotic properties
  –Lim et al, Mol Nutr Food Res, 17:72, 2005

synbiotics

- combination of pro- and prebiotics in one product
  – Selma et al, Food Funct, 7:1769, 2016

use of probiotics for GI conditions

- meta-analysis of 74 randomized, placebo controlled studies showed significant efficacy of probiotic treatment for:
  - antibiotic associated diarrhea
  - Clostridium difficile disease
  - Helicobacter pylori positive
  - pouchitis
  - irritable bowel syndrome
  - infectious diarrhea
- not effective for necrotizing enterocolitis or traveler's diarrhea
- magnitude of effect varied by study

Ritchie and Romanuk, PlosOne 2012

use of probiotics for obesity treatment?

- obesity and obesity-related metabolic disorders characterized by altered gut microbiome composition and function.
- altering gut microbial communities can reduce adiposity in rodents.
- lack of consistent data in humans that probiotics reduce or prevent adiposity.

Davis, *Nutr Today*. 2016; 51:167

probiotics and CVD risk management

- systematic review of 13 studies of type 2 diabetic patients:
  - 10 RCTs, 1 single-blinded and 2 crossover trials
  - 352 intervention; 358 placebo
- showed efficacy of probiotic treatment in:
  - reducing total- and LDL-cholesterol and triglycerides
  - reducing systolic and diastolic blood pressure
- no effect on HDL-cholesterol
- treatments and magnitude of effect varied

Hendijani & Akbari, Clin *Nutr*. 2018; 37:532

probiotic mechanisms of action

effects on GI symptoms

- modification and stabilization of gut community
- reduction in gut permeability
- stimulation of non-specific and specific immune responses

effects on markers of metabolic disorders

- reduction in cholesterol absorption
- alterations in microbial bile acid metabolism and reabsorption

Ritchie and Romanuk, *PlosOne* 2012
Hendijani & Akbari, *Clin Nutr*. 2018; 37:532

potential contributions of fermented foods

fermented foods as probiotics?

legumes • grains • vegetables • fruit • milk • fish • meat

North America
Sauerkraut
Relishes
Sourdough breads
Cultured milk
Mikyuk (Alaska)

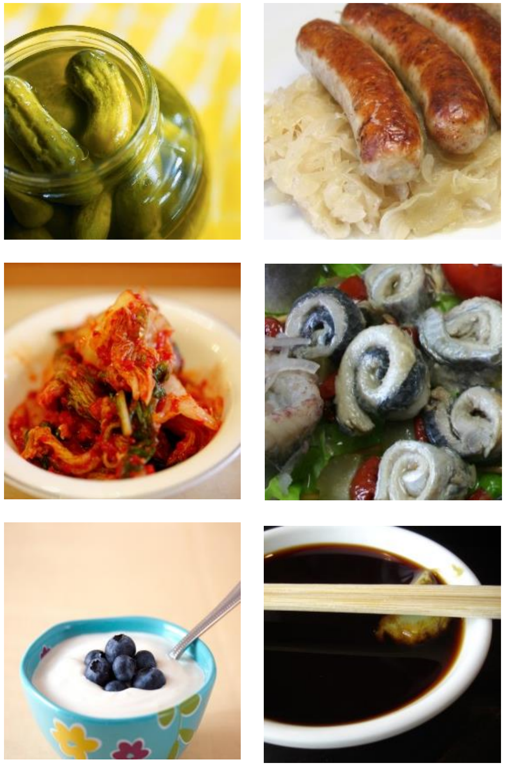
Western Europe & Russia
Sauerkraut
Sourdough breads
Salami
Cultured milk

Africa & Mid-East
Grain gruels
Injera (Ethopia)
Kumi
Kefir

Japan, Korea, China
Kimchi
Natto
Soy sauce
Fish
Fish sauces

Central & South America
Curdito
Chocolate

SE Asia
Paneer
Tempeh
(Indonesia)
Idli (India)
Fish sauces



Steinkraus, KH (ed), *Handbook of Indigenous Fermented Foods*, 1983.

benefits of fermented foods

- preserve foods between time of harvest and consumption
- render foods resistant to microbial spoilage and development of food toxins
- enrich the diet through development of diverse flavors, aromas, and textures in food substrates
- enrich biologically with protein, essential amino acids, essential fatty acids, and vitamins
- reduce content of antinutrients (e.g., phytate, lectins)

Steinkraus, KH (ed*), Handbook of Indigenous Fermented Foods*, 1983.

fermented milk products

- source of probiotics, mostly *Lactobacillus* and *Bifidobacterium*
- yogurt delivers a large number of live active cultures to GI tract.
- yogurt consumption:
  - positively associated with improved glucose metabolism
  - inversely associated with risk of type 2 diabetes
- randomized controlled interventions are needed to unequivocally show that yogurt and its components can prevent the onset of diabetes

Donovan and Salas-Salvadó, *J Nutr* 147:1449S, 2017
Salas-Salvadó et al, *J Nutr* 147:1452S, 2017 • Wen and Duffy, *J Nutr* 147:1468S, 2017

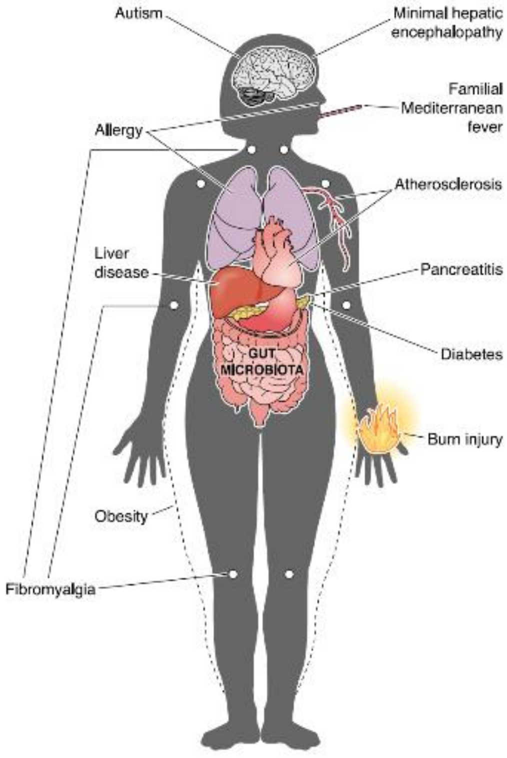
impact of the gut microbiome on human health

microbes and disease

- microbes as infectious agents
  - account for ~20% of cancers worldwide
  - cervical, liver and gastric cancers
- microbes as modifiers of physiology
- microbes as modifiers of exposures
  - metabolize dietary constituents, drugs, carcinogens
  - affecting energetics and obesity

microbiome dysbiosis and disease

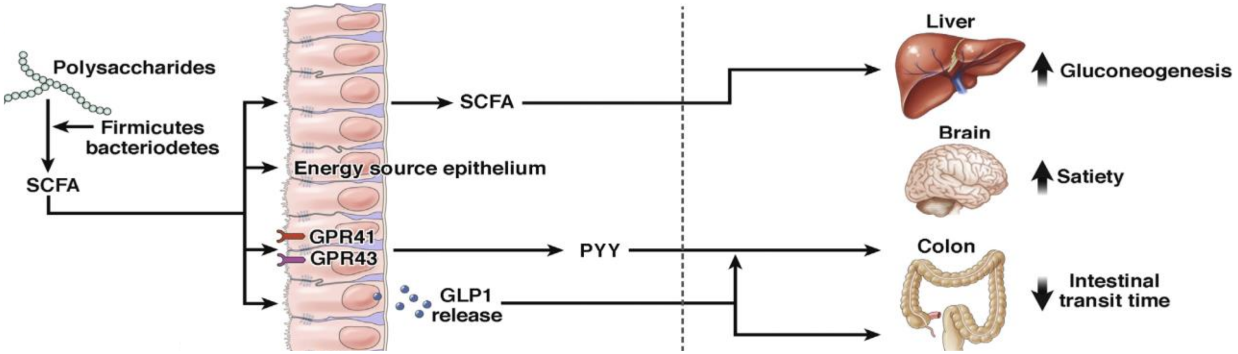
- autism
- autoimmune diseases
- colon cancer
- hepatic encephalopathy
- irritable bowel syndrome (IBS)
- inflammatory bowel disease (IBD)
- metabolic syndrome/diabetes
- non-alcoholic fatty liver disease
- obesity



Sekirov I et al, *Physiol Rev*, 2010

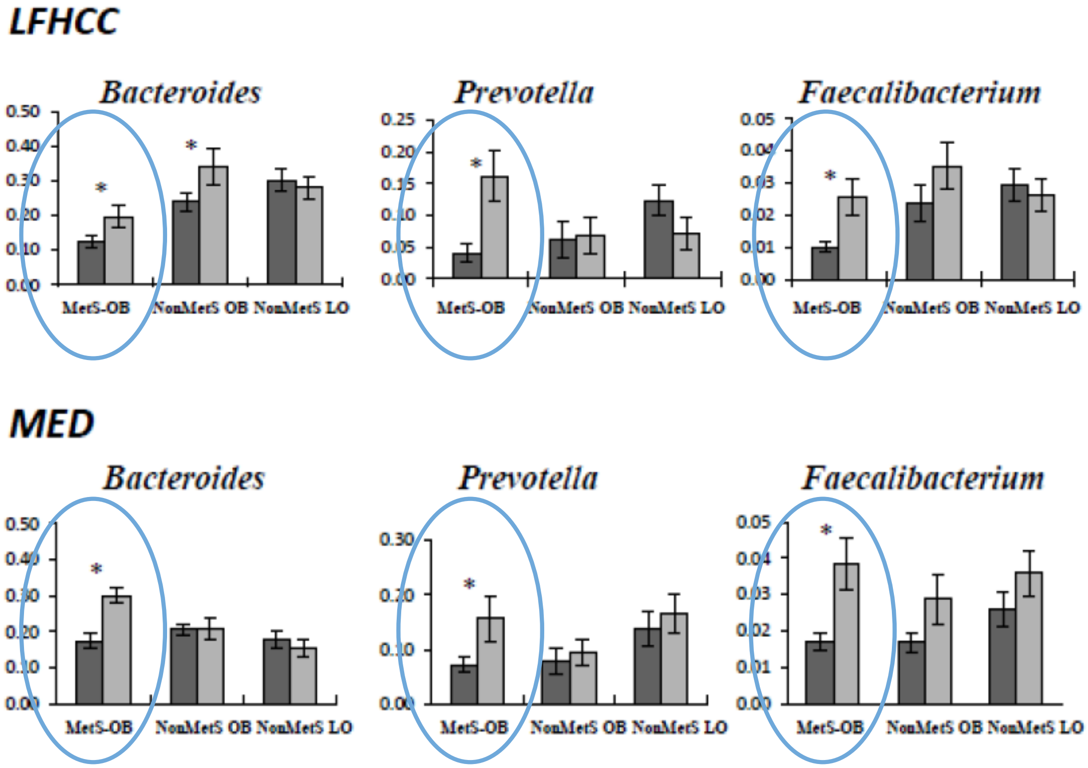
role of the gut microbiome in obesity-related metabolic dysfunction

gut lumen **blood** **end organs**



Bouter et al, *Gastroenterol*, 2017;152:1671.

low-fat and Mediterranean diets restore microbiota in obese patients with metabolic syndrome



- 106 subjects in CARDIOPREV
  - 33 obese with MetS
  - 32 obese
  - 41 non-obese
- LF and MED diets increased key genera in MetS-OB group.

diet did not alter microbiota in non-obese and obese groups

C Haro et al, *Mol Nutr Food Res*, 2018, in press.

things to consider when exploring microbiome and disease literature: study design

observational, association studies:

- prospective cohort study vs case-control?
- effects of confounding?

interventions:

- parallel arm vs crossover?
- placebo or other relevant control?
- long enough intervention period?
- well-described intervention?
- participant adherence?

issues of association vs causation

things to consider:
study population and sampling

study sample

- large enough and adequately powered?
- well-defined population?
- sufficient meta-data?
- any issues of bias?

how was microbiome assessed?

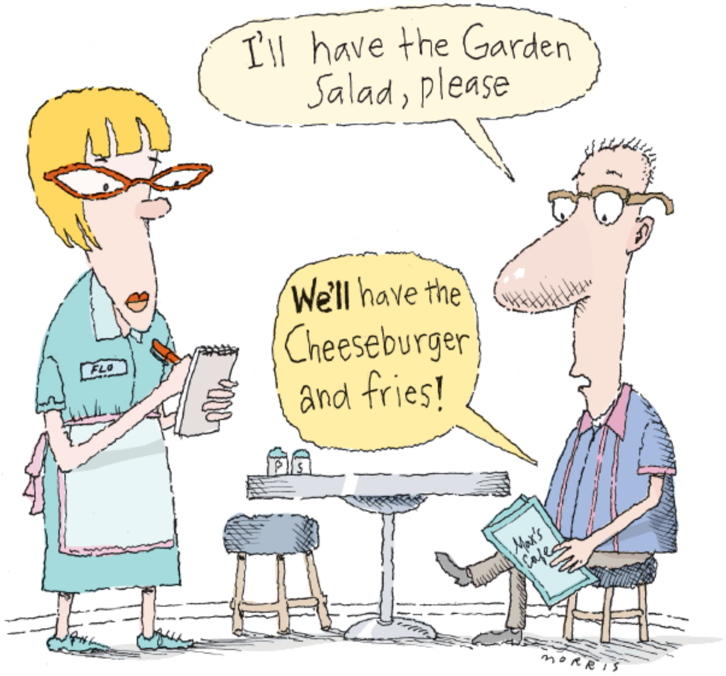
- phylum, genus, or species level?
- is there sufficient granularity to test the hypothesis?

limited insight into magnitude of risk or benefit

- statistically significant, but is it clinically relevant?

consumer takeaways

recommendations for gut microbiome health: the microbial perspective



- a young field of research
- a lot we still don't know

general recommendation:

- avoid indiscriminate use of antibiotics
- make food choices to maintain gut microbiome diversity and function

dietary recommendations for gut microbiome and human health

choose a healthy eating pattern including:

- a variety of vegetables from all the subgroups
- fruits, especially whole fruits
- grains, at least half of which are whole grains
- a variety of protein foods, including … dairy, legumes, nuts, and seeds
- oils from plant sources

2015-2020 Dietary Guidelines

summary

- diet influences the gut microbiome and its impact on the host
- large variation in gut microbiome across individuals and diet-induced changes often small
- microbial production of bioactive substances varies greatly across individuals
- substantial work needed to establish causal relationships and therapeutic potential in humans

questions?

Thank you!

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