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Národní institut
virologie a bakteriologie

The human microbiome and physiological microbiota

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and Motol University Hospital

Microbiology II (3rd year)
2025/2026

Outline

- **The gut microbiome**
 - General introduction and current state of knowledge
 - **Commercial testing** – is it worthwhile or not?
 - **How to care for the microbiome:** fibre and its impact on health
 - **Antibiotics** and their negative impact on the microbiome
- **Physiological microbiota**

Interest is growing

RESEARCH ARTICLE

Microbiome research in general and business newspapers: How many microbiome articles are published and which study designs make the news the most?

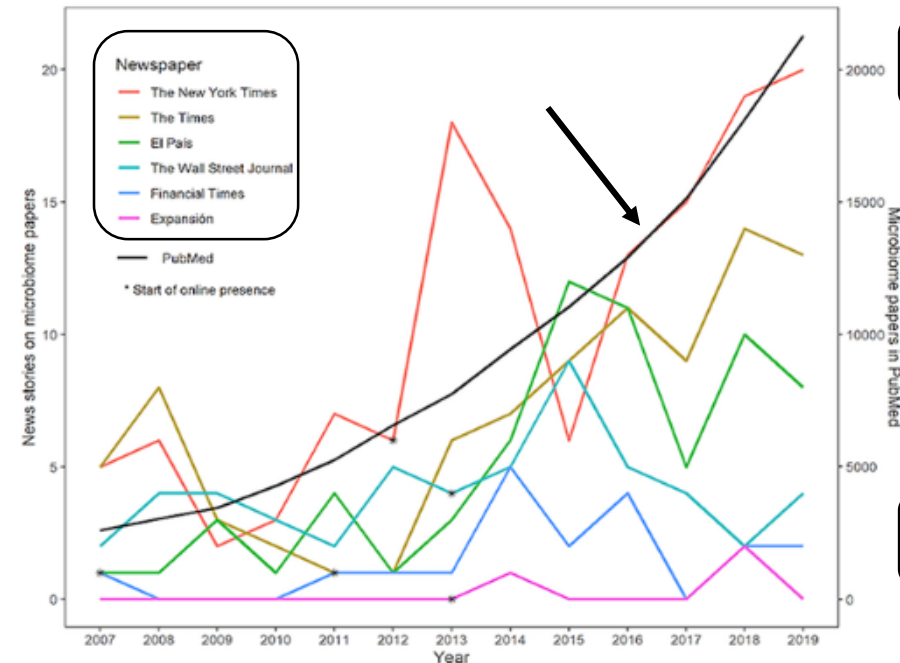
Andreu Prados-Bo^{1,2*}, Gonzalo Casino^{1,3*}

¹ Department of Communication, Pompeu Fabra University, Barcelona, Spain, ² Blanquerna School of Health Sciences, Ramon Llull University, Barcelona, Spain, ³ Iberoamerican Cochrane Centre, Biomedical Research Institute Sant Pau (IIB Sant Pau), Barcelona, Spain

- The professional community takes note
- The general public is taking notice

You should too!

A



2019: approx.
20,000/year

2007: approx.
2,000/year

Prados, Casino: Microbiome research in general and business newspapers: How many microbiome articles are published and which study designs make the news the most?
PLOS One. 2019. 10.1371/journal.pone.0249835

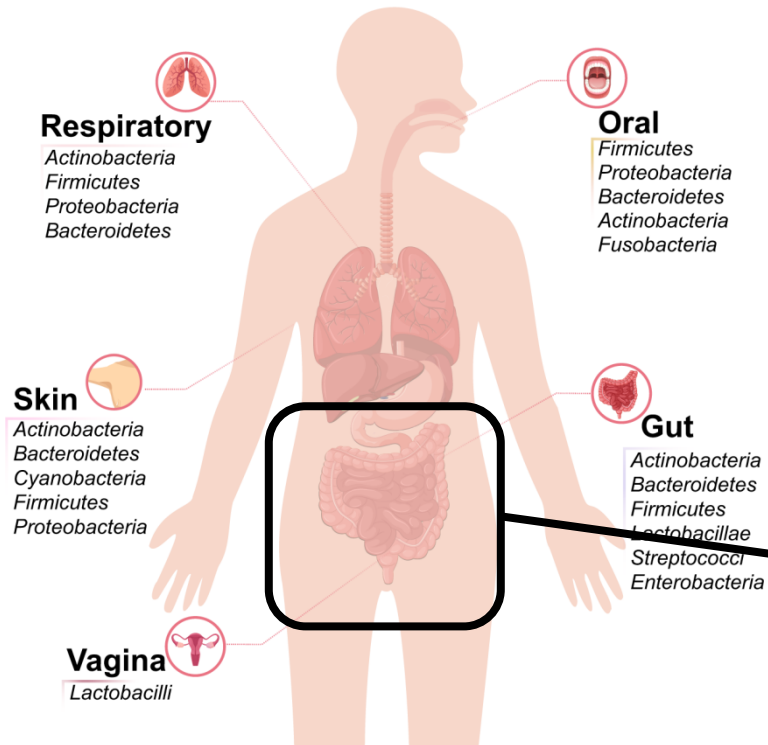
A necessary introduction to the gut microbiome

What it is, what it consists of, and what influences it

Microbiome

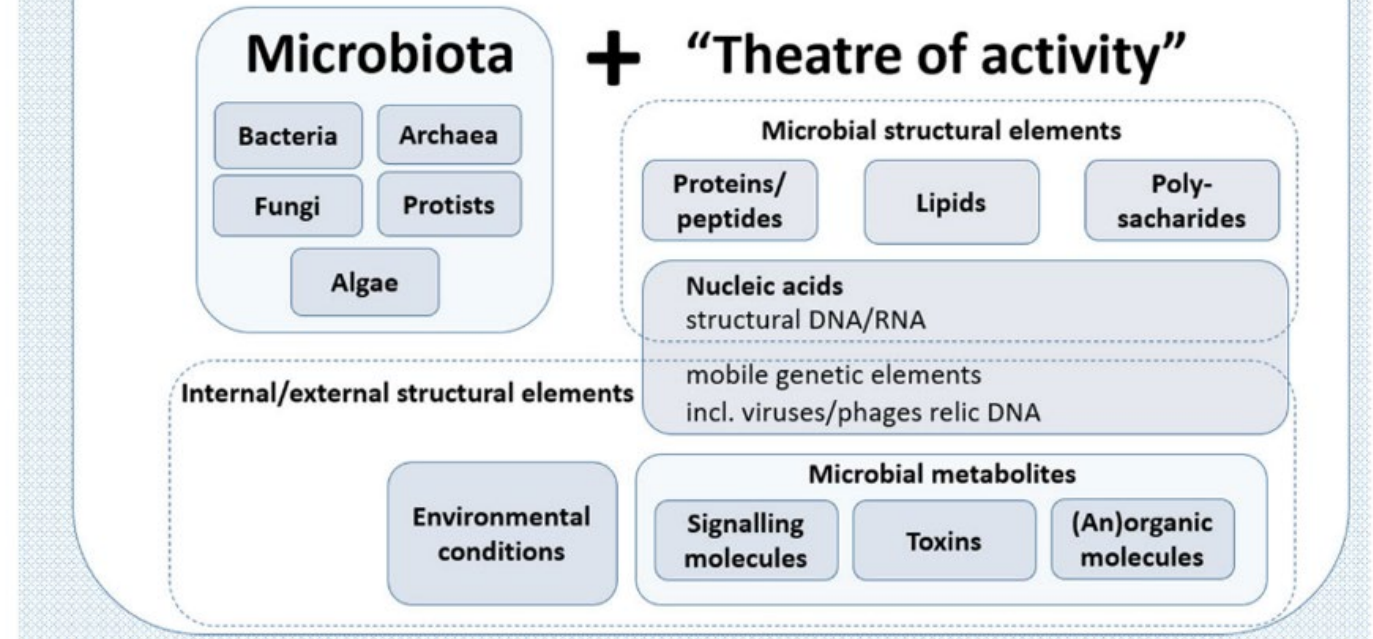
Microbiome = a characteristic microbial community that inhabits a specific, rationally defined habitat with typical physical and chemical conditions

Berg et al, *Microbiome*, 2020

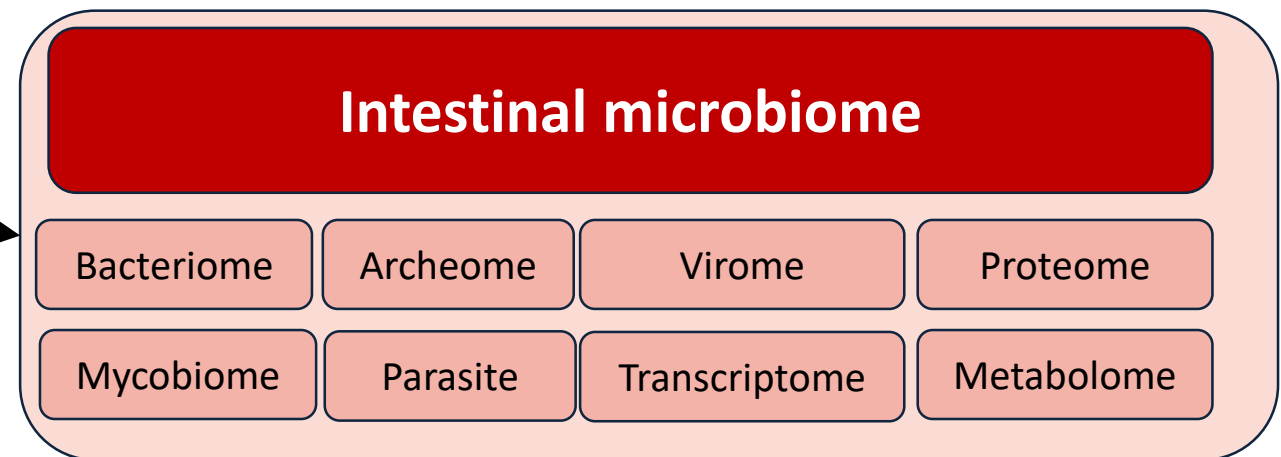


Hou et al, *Sig Transduct Target Ther*, 2022

Microbiome



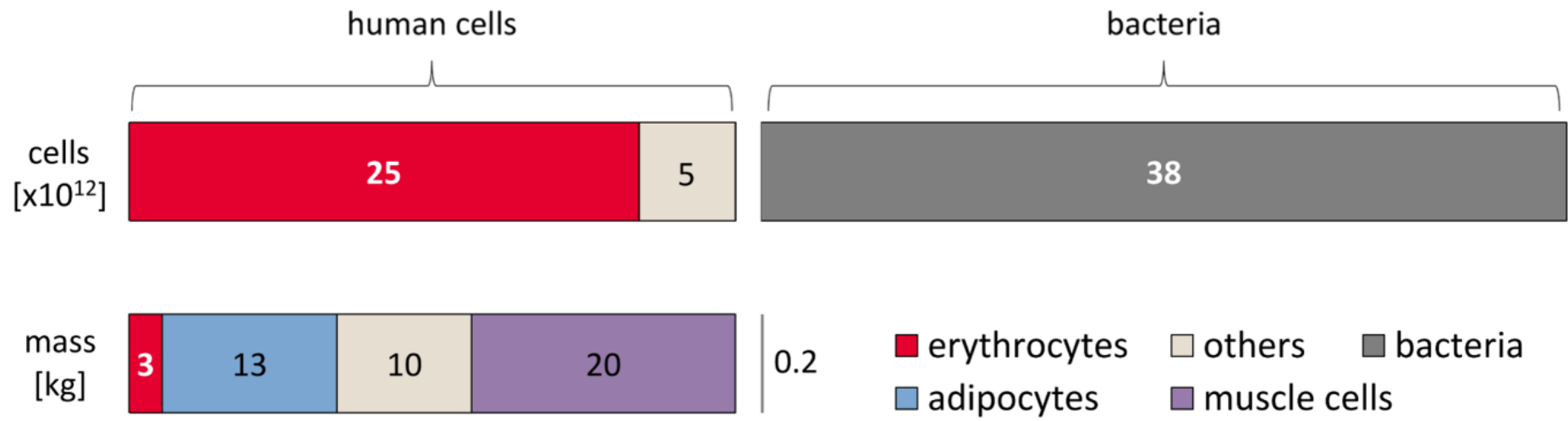
Berg et al, *Microbiome*, 2020



Terminology

Term	Definition
Microbiome	A characteristic microbial community that inhabits a specific, rationally defined habitat with typical physical and chemical conditions.
Microbiota	A collection of living microorganisms within a given microbiome
Probiotics	Living microorganisms that, when administered in sufficient quantities, confer a health benefit on the host.
Prebiotics	Food components that serve as "energy sources" for beneficial intestinal bacteria.
Synbiotics	Food supplements containing a combination of probiotics and prebiotics.

Are we more humans or microbes (bacteria)?



Sender et al, PLOS, 2016

	Number of cells	Number of genes	Mass
Human 	30 trillion (3.0×10^{13})	20-25 thousand (2.0×10^4)	70-100 kg
Microbes 	38 trillion (3.8×10^{13})	2-20 million ($2.0 \times 10^6 - 2.0 \times 10^7$)	0.2 kg
	1.3 times more bacteria 	100 times more bacteria 	350-500 times more human 

Physiological functions of the gut microbiome

Defence against
infection

Digestion

Immune system

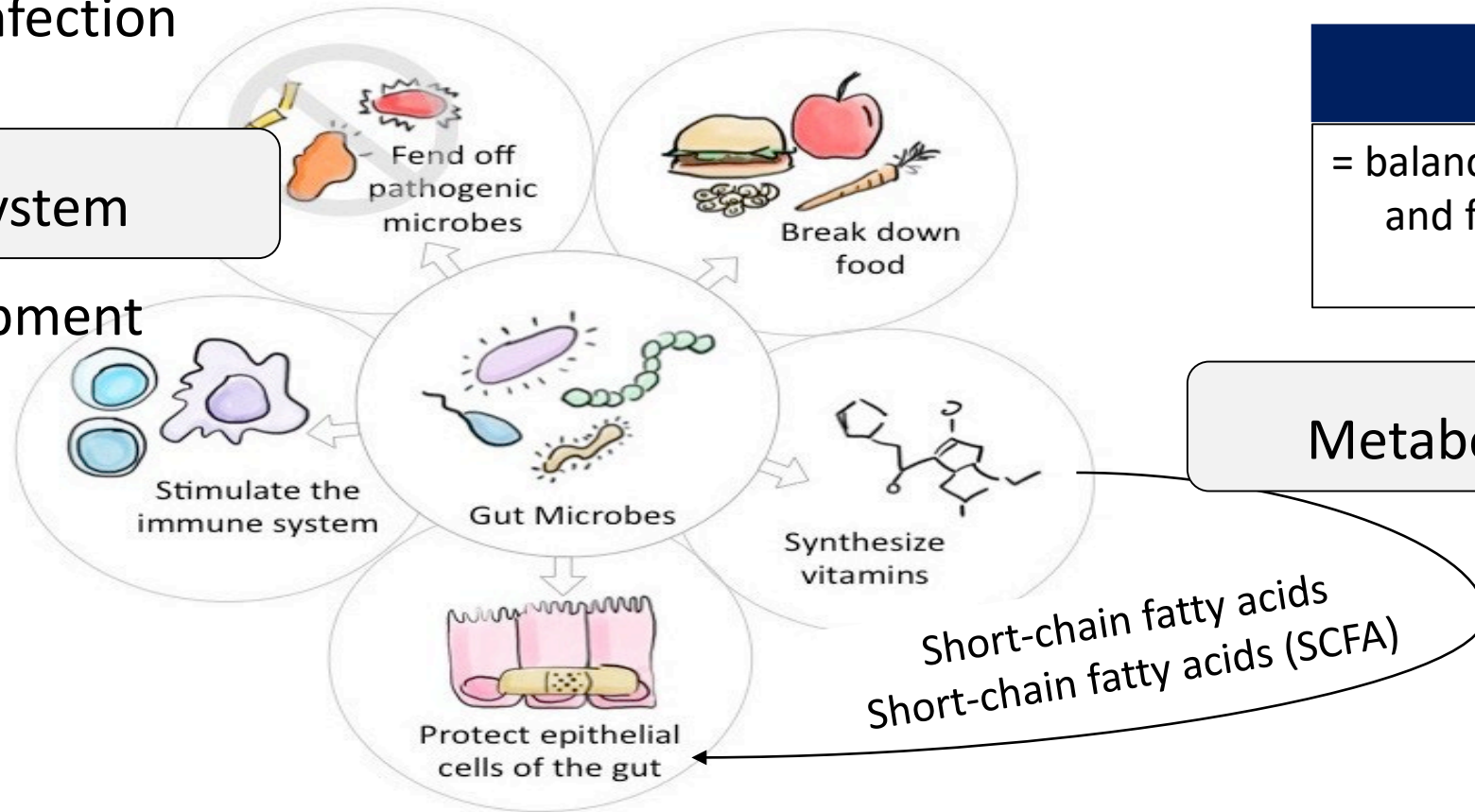
development

EUBIOSIS

= balance in the composition
and function of the gut
microbiome

Metabolism

Intestinal wall integrity

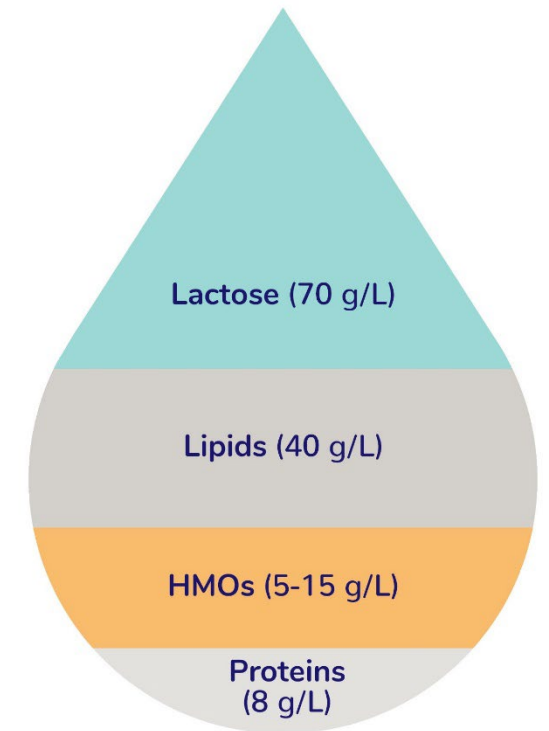


Breast milk and microbiome nutrition

The crucial importance of HMO (human milk oligosaccharides)

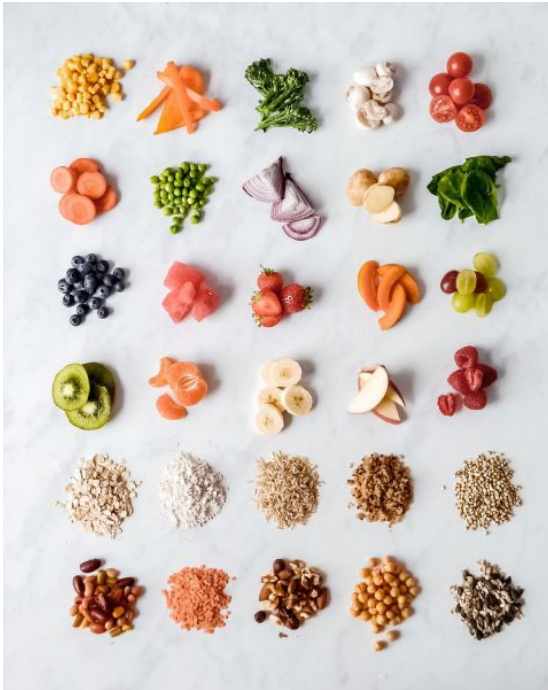
- The third most common component, indigestible for infants but **an important food source for bacteria**:
 - 1) **Bifidobacteria**. They prevent colonisation by other pathogenic microbes from the mother's nipple, such as *Staphylococcus*.
 - 2) **Lactobacilli**. These are able to break down sugars in HMO, which can then be used by the infant as a source of energy.

HMOs are the 3rd largest solid component in human milk



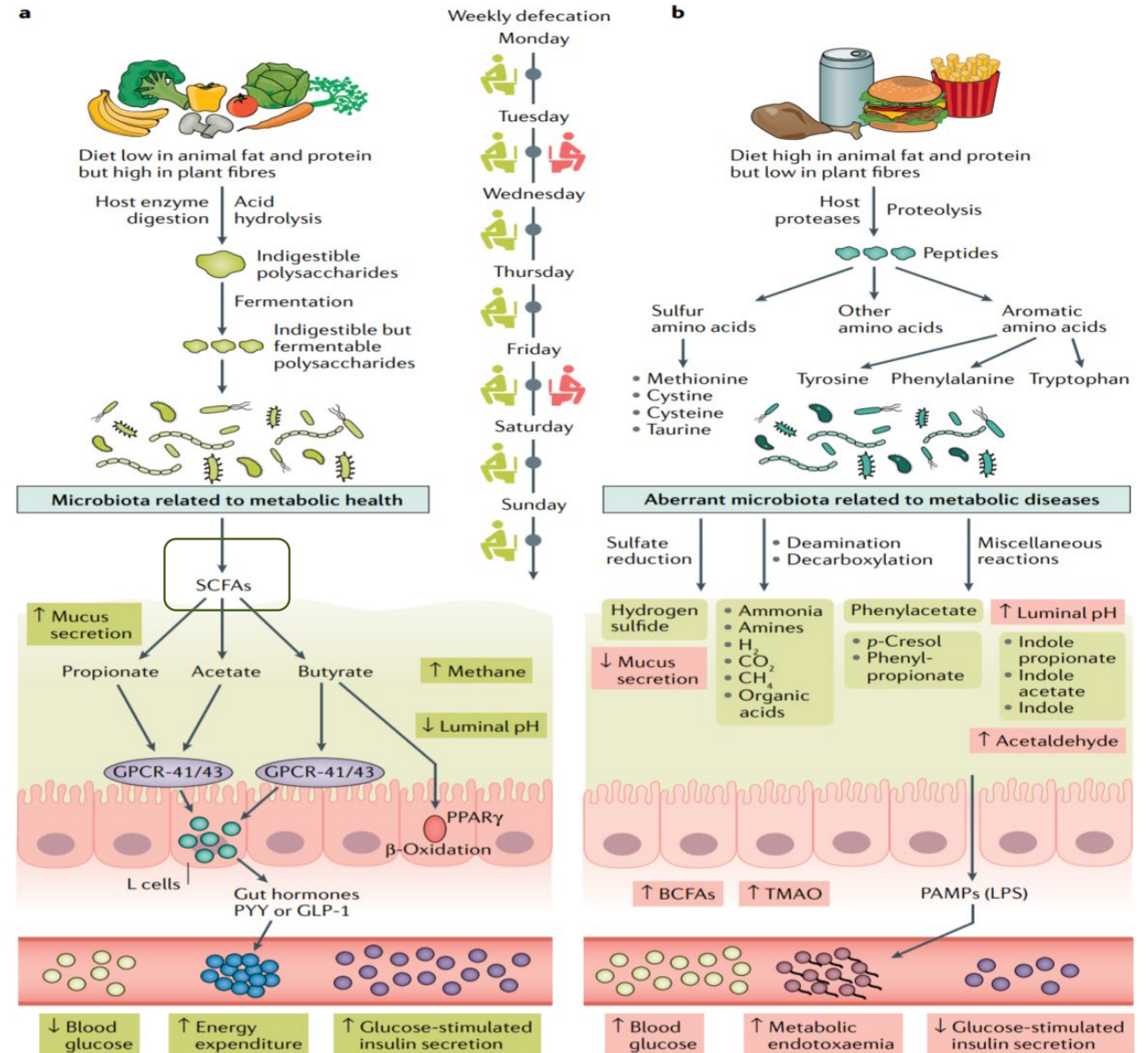
[nestle.com](https://www.nestle.com)

What exactly is a high-fibre diet?



"Thirty different plants per week"

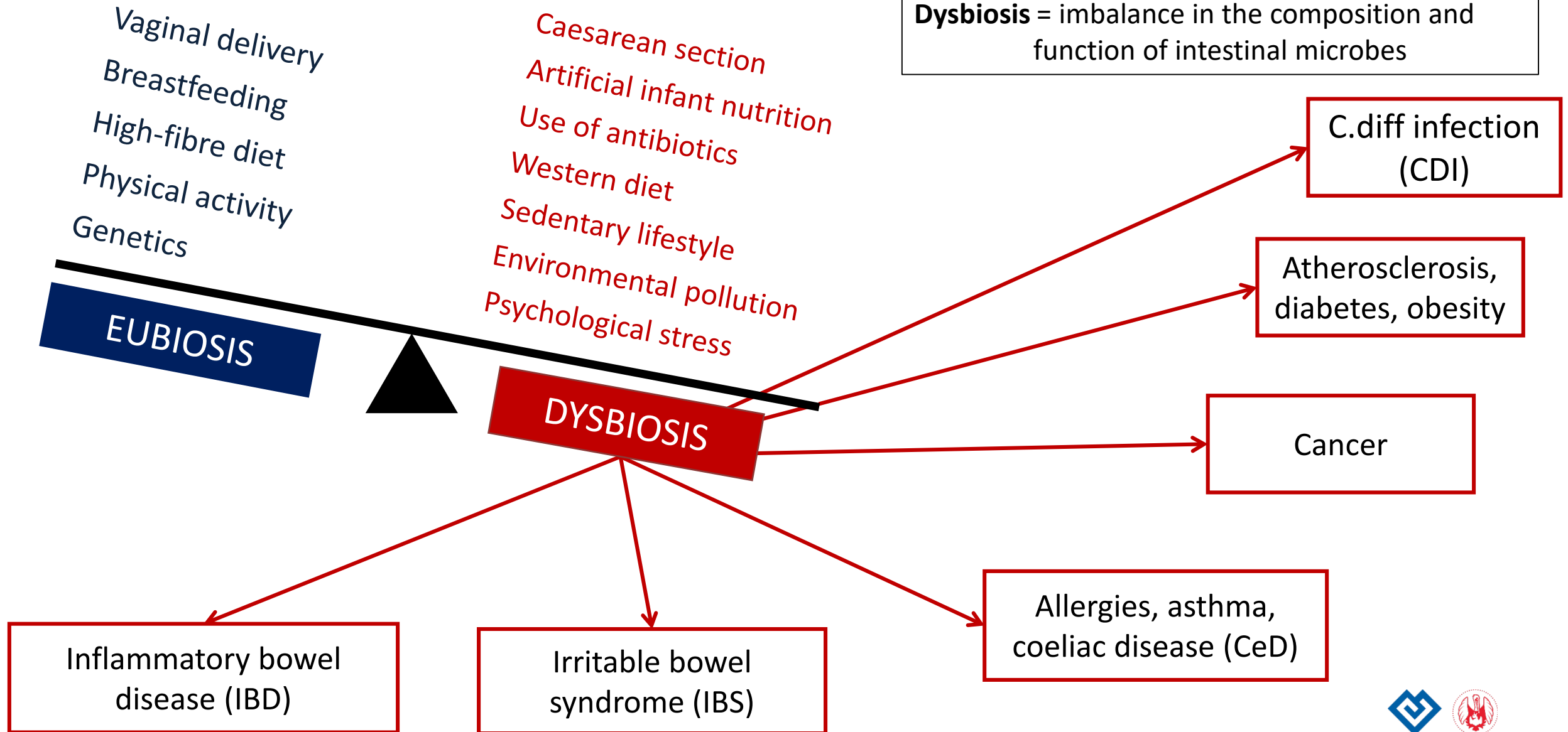
(Knight et al, American Gut Project, 2012)



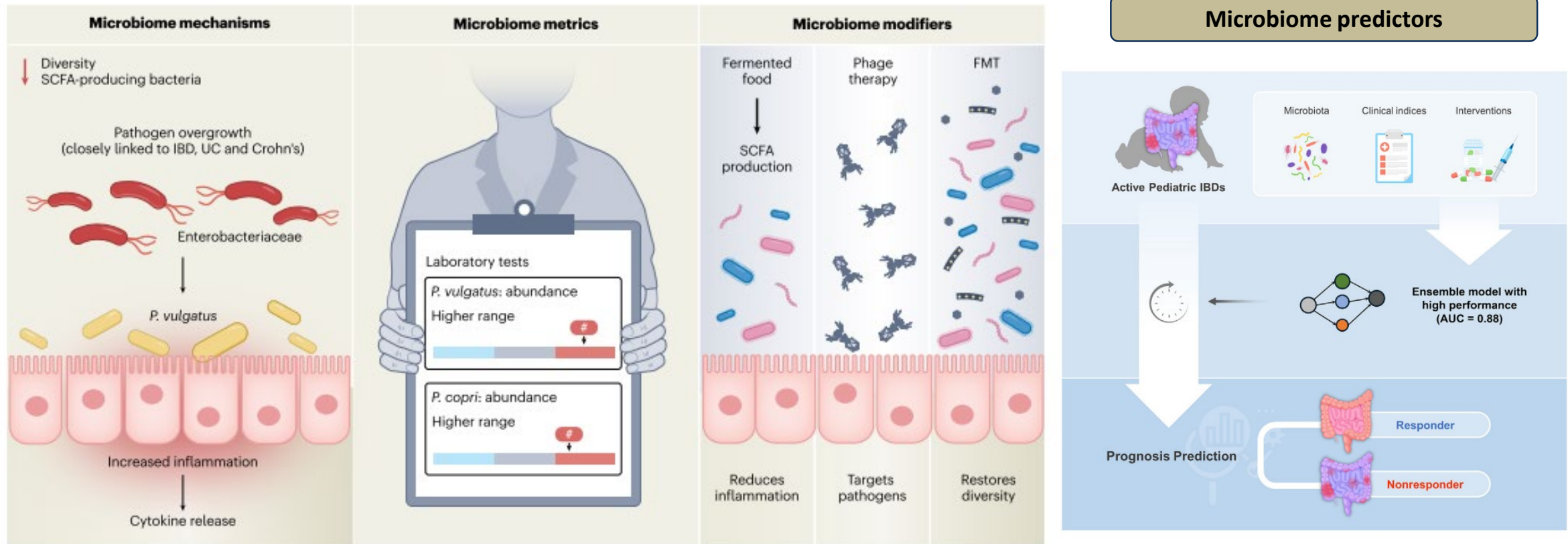
Fan & Pedersen; *Nature Reviews Microbiology*, 2020

Influence on the composition of the gut microbiome

Dysbiosis = imbalance in the composition and function of intestinal microbes



How this translates into practice – an example on IBD



Understanding the mechanism

Reduced diversity and proportion of SCFA producers

Finding measurable metrics

Representation of *Phocaeicola vulgatus* (neg) and *Prevotella copri* (pos)

Find therapeutic tools

Diet, phage therapy or FMT

Predict anything

Response to treatment based on composition

How the microbiome is analysed

A bit boring, but it helps to understand the whole thing

Methods for studying the gut microbiome

Stool sample collection

At patients' homes

Storage:

- short term at -18°C
- long-term at -80°C



DNA extraction



16S/18S rDNA profiling

PCR for 16S/18S rDNA → ←

Massively parallel
amplicon sequencing



Comparison of ASV/OTU with reference
database

Evaluation of **taxonomy**

Metagenomic sequencing

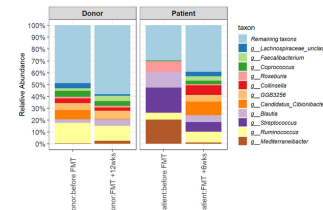
Random fragments,
ALL DNA



Sequencing
Genome assembly

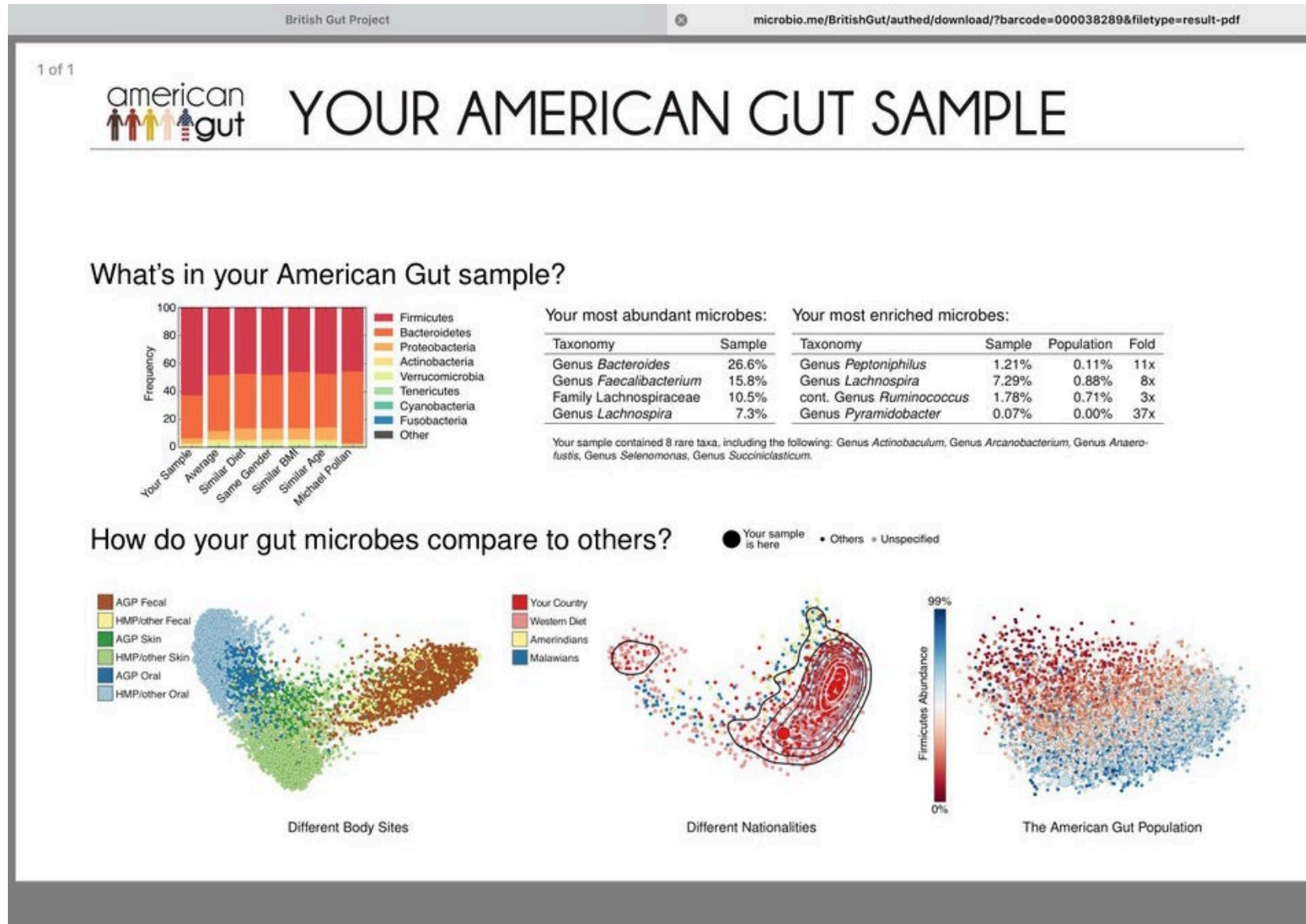
Evaluation of both taxonomy (more in-depth)
and prediction of **functional capabilities of
the microbiome**

Comprehensive
bioinformatic analysis
and evaluation of
outputs

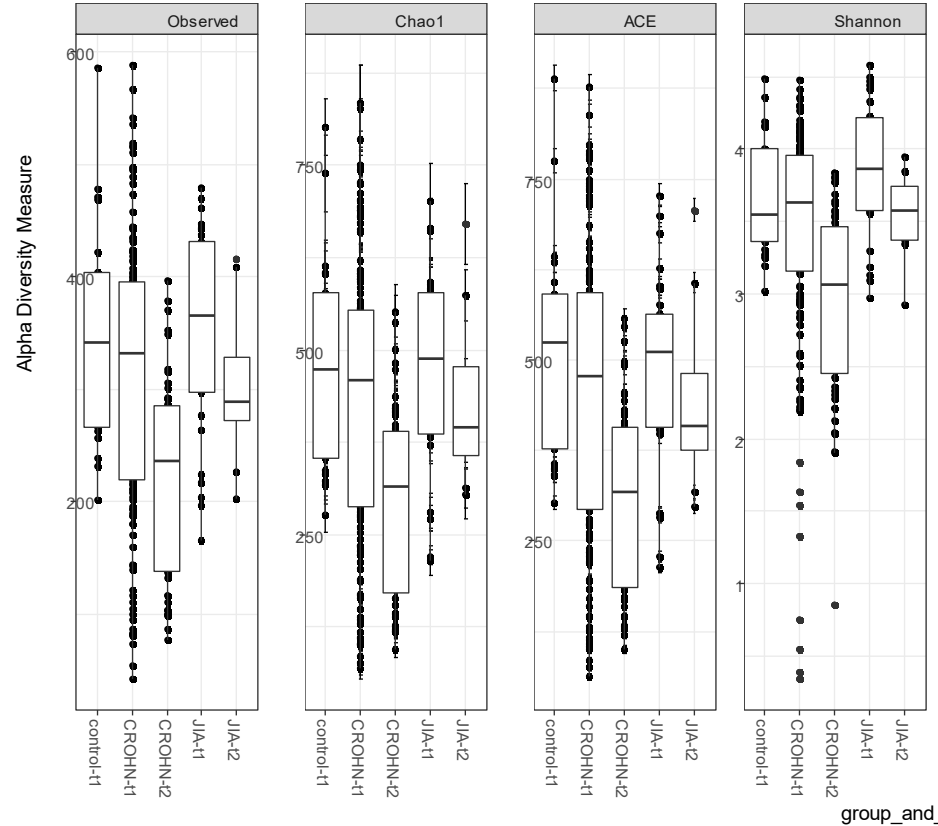


More cool!

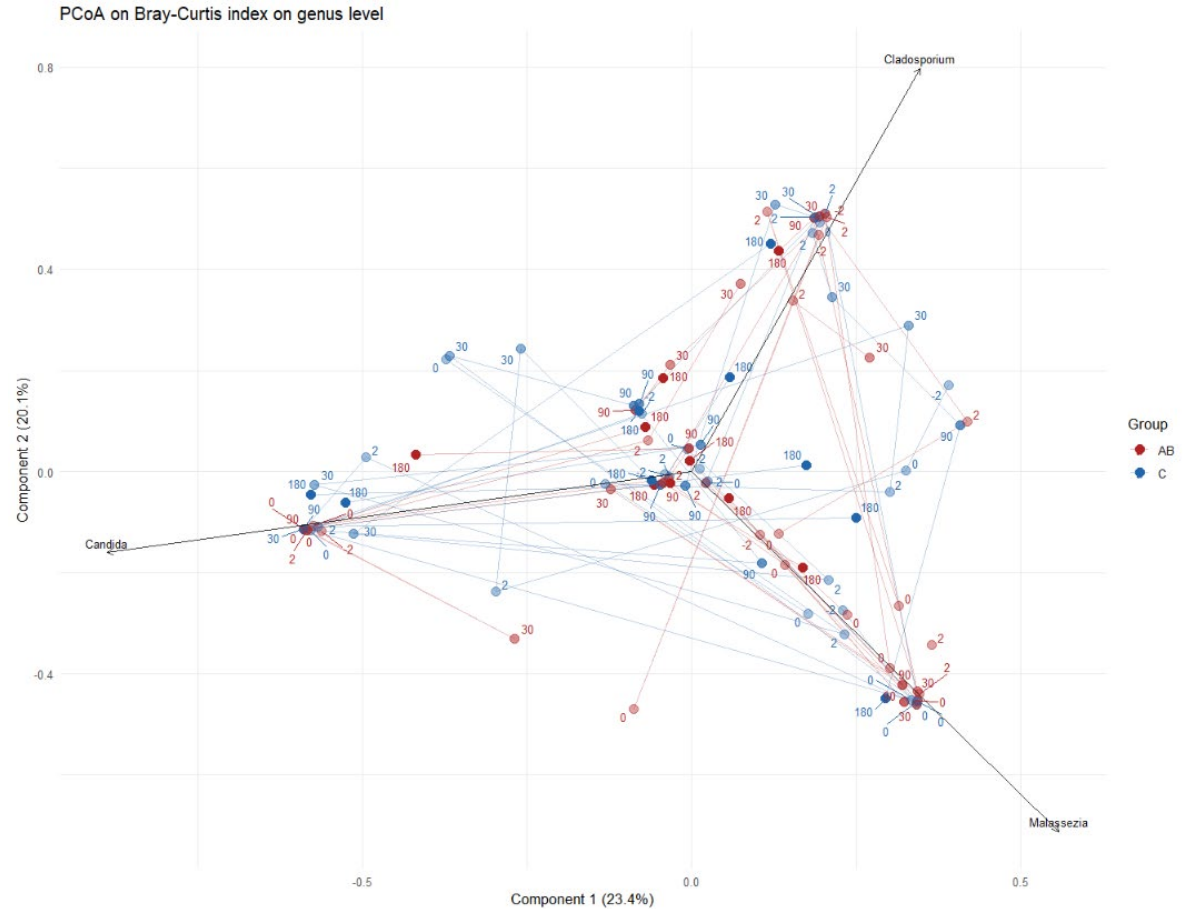
What the result might look like



Diversity - alpha and beta



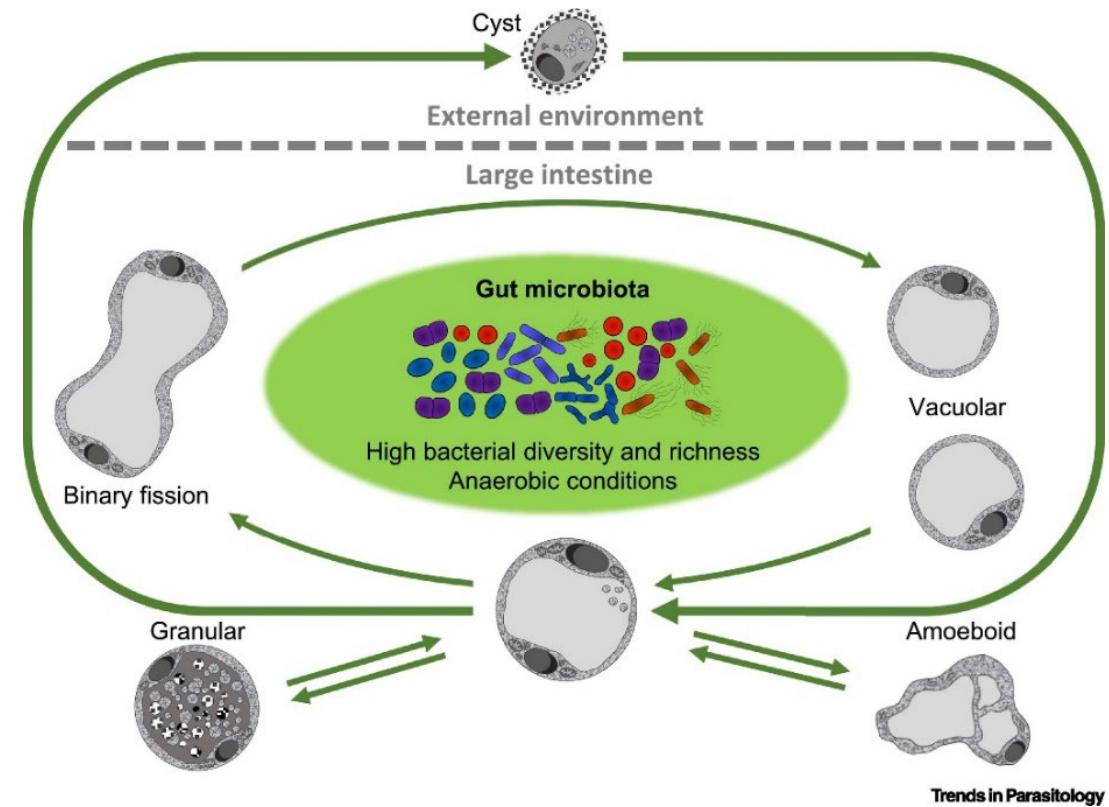
Alpha = within one sample



Beta = between samples

Non-bacteria: *Blastocystis*

- The most abundant eukaryote in the human gut ¹⁻³
- **Marker of high bacterial diversity** ^{4,5}
- Prevalence varies
 - Higher in developing countries (40-100%) ⁶⁻⁸
 - Lower in industrialised countries (7-50%) and intestinal diseases (up to 5%) ⁹⁻¹²
- **Marker of cardiometabolic health**, more common in people on a plant-based diet ¹³



Stensvold, Trends in Parasitology, 2020

1)Tito. *Gut*. 2019; 2) Andersen. *FEMS Microbiol Ecol*. 2015; 3) Rostami. *Parasitol Res*. 2017; 4) Clark. *Adv Parasitol*. 2013; 5) Cinek. *Parasit Vectors*. 2021; 6) Poulsen. *Am J Trop Med Hyg*. 2016; 7) Mohammad. *Asian Pac J Trop Med*. 2017; 8) Oliveira-Arbex; *Infect Genet Evol*. 2018; 9)Wawrzyniak. *Ther Adv Infect Dis*. 2013; 10) Stensvold. *Parasitol Int*. 2016; 10) Bart. *BMC Infect Dis*. 2013; 11) El Safadi. *BMC Infect Dis*. 2016; 11) Scanlan. *Infect Genet Evol*. 2016. 11) Scanlan. *FEMS Microbiol Ecol*. 2014. 12) Lhotska. *Front Cell Infect Microbiol*. 2020; 13) Piperni et al. *Cell*. 2024

What is a "good and bad" composition?

GOOD

High alpha diversity (300-1000 species)

Anaerobic environment (e.g. very few *Proteobacteria*)

More SCFA producers

Blastocystis positive

Healthy microbiome?

BAD

Low alpha diversity (less than 100 genera)

Many facultative anaerobes (e.g. more *Proteobacteria*)

Few SCFA producers

Blastocystis negative



The concept of a healthy microbiome **does not yet exist**

[ČMS](#) ▾[Pro odborníky](#) ▾[Pro pacienty a laiky](#) ▾[Podcasty](#)[Pro členy](#)[English](#) ▾

A jaké by tedy měl mít zdravý člověk složení mikrobiomu?

NEVÍME – a neví to zatím nikdo, ani komerční subjekty, které nabízejí, že Vám mikrobiom opraví!

Vědci mají jistě všeobecnou představu o složení mikrobiomu, ale pro konkrétního člověka v konkrétní životní situaci to definovat zatím nelze.

Vezmeme-li v potaz, že počet genů mikroorganismů ve střevním traktu je 100-150x větší, než počet genů v genomu člověka, a zároveň je genetická informace mikroorganismů vysoce individuální a variabilní, odpovídá to na otázku, proč je tak těžké mikrobiom studovat a proč nejsou výsledky více uplatňované v praxi. Zatím je prozkoumána pouze část mikroorganismů, u nichž víme, jaké živiny spotřebovávají a jaké látky produkují. Mnohé jsou prospěšné, některé mohou za určitých podmínek organismu škodit, o jiných zatím nevíme téměř nic.

V některých případech lze skutečně říct, že konkrétní složení mikrobiomu je nezdravé (např. převaha patogenních kmenů bakterie *Clostridioides difficile* způsobujících úporné průjmy), ale nikdo dosud nedokázal přesně definovat „zdravé“ nebo „správné“ složení mikrobiomu.

www.mikrobiom-cms.cz

Where is the knowledge about the microbiome?

- It is getting closer and closer to practical application. We can point to:
 - microbial metrics that can help diagnose diseases ¹
 - or indicate the success of treatment (currently only for FMT) ²

Not yet in common use, large studies are still ongoing (see below)

"The future use of personalised microbiome interventions that take into account individual microbiome composition and realistic lifestyle changes **will help** bridge the gap between research and practice **in the future.**" – Gilbert et al., *Nature Medicine*, 2025

nature medicine

Review article

<https://doi.org/10.1038/s41591-025-03615-9>

Clinical translation of microbiome research

Received: 27 October 2024

Accepted: 26 February 2025

Published online: 11 April 2025

 Check for updates

Jack A. Gilbert^{1,2,3}✉, Meghan B. Azad^{4,5,6}, Fredrik Bäckhed^{7,8}, Martin J. Blaser^{6,9}, Mariana Byndloss^{10,11}, Charles Y. Chiu^{12,13,14}, Hiutung Chu^{3,15,16}, Lara R. Dugas^{17,18}, Eran Elinav^{19,20}, Sean M. Gibbons^{21,22,23,24}, Katharine E. Gilbert³, Matthew R. Henn²⁵, Suzanne L. Ishaq^{26,27}, Ruth E. Ley²⁸, Susan V. Lynch²⁹, Eran Segal³⁰, Tim D. Spector^{31,32}, Philip Strandwitz³³, Jotham Suez³⁴, Carolina Tropini^{5,35,36}, Katrine Whiteson³⁷ & Rob Knight^{1,3,38,39,40}

- 1) Schmartz, G. P. et al. Nat. Commun. 2024
- 2) Weingarden, A. et al. Microbiome. 2015.

"The microbiome is the key to health," and the key to health is the diversity of plant foods.



The question is: "Can I have my microbiome tested commercially?"

The answer: YES!

Question: "Can I test my microbiome and obtain valid information that will help me?"

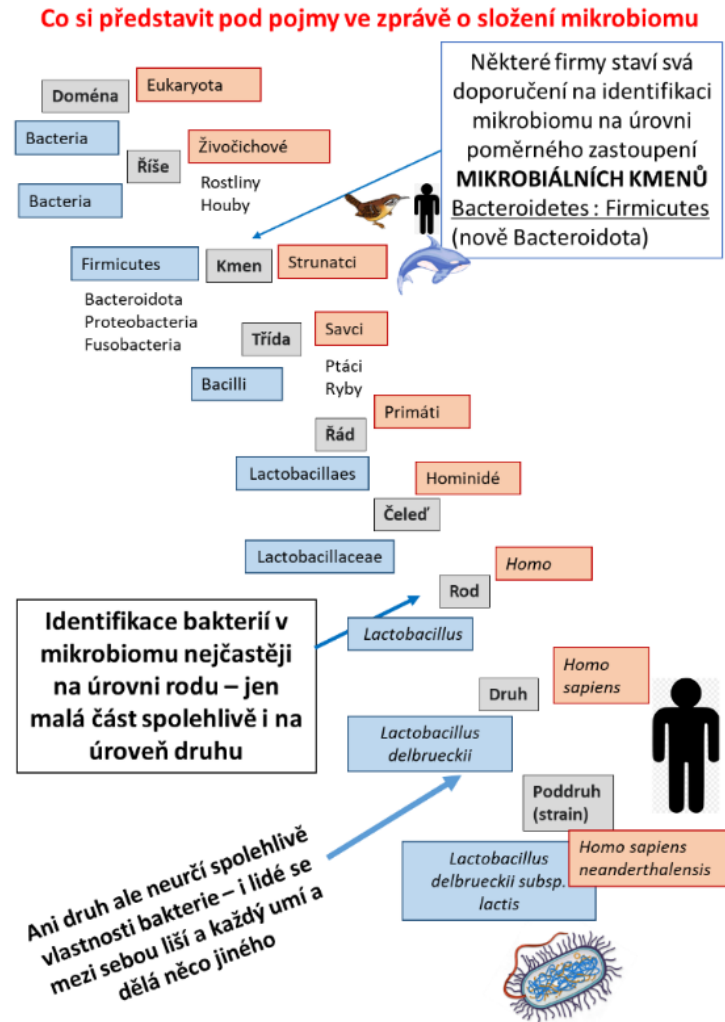
Answer: No.

And why?

What are the problems? I.

Insufficient depth of taxon identification

- Information about the representation of the main "groups" – usually phyla and their ratios (Firmicutes/Bacteroidetes)
- **The Firmicutes/Bacteroidota (F/B) ratio** has long been considered a simple indicator of health or dysbiosis, but **the current scientific consensus strongly questions** this indicator.



Obr. 1 Co si představit pod pojmy ve zprávě o složení mikrobiomu

What are the problems II.

Potentially incorrect interpretation of the function of the microbiome

- Insufficient depth – see point 1.
- The mere presence of a certain bacterium does not imply specific abilities, and these in turn do not imply that a process, e.g. the production of certain substances, is actually taking place (see Fig.).

Multiomics – the need for metabolomics

Dysbiózu ve střevě si lze představit jako dům po zásahu tornáda



Jak dům opravit?

Bude potřeba mnoho řemeslníků, např. zedník.

Zedník = člověk (*Homo sapiens*)

ALE Ne každý *Homo sapiens* je ale zedník

Homo sapiens
- chemik



Homo sapiens
- doktor



Homo sapiens
- zedník



Zedník = umí stavět dům
= má tu schopnost
ALE – někdy to nedělá

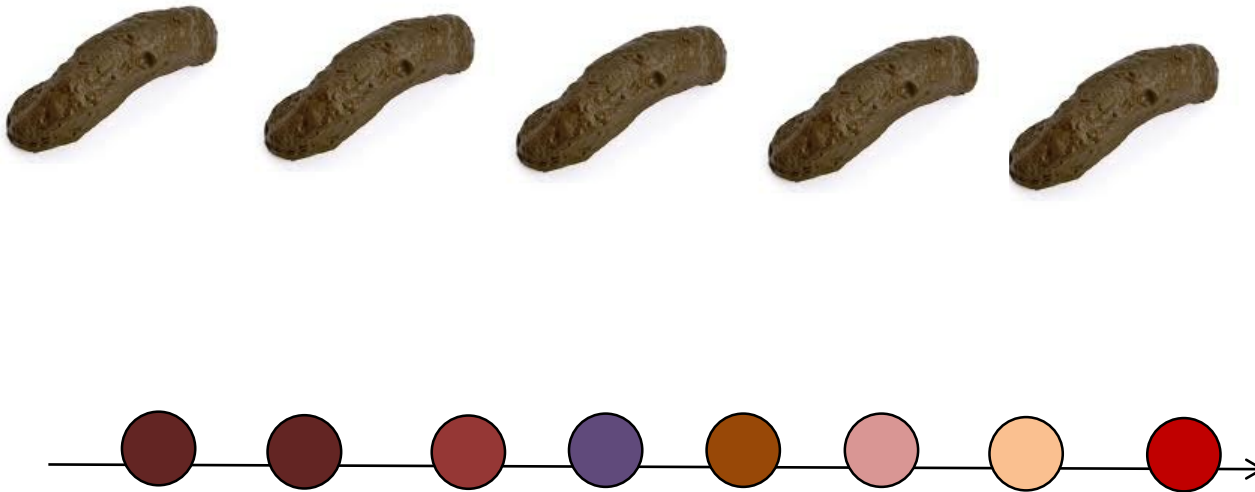


Stejně tak např. ne každá bakterie *Lactobacillus reuteri* má potřebné schopnosti právě pro vaše střevo, a i když ty schopnosti bude mít, za určitých podmínek je nemusí projevit.

What are the problems III.

Conclusions based on a single sample

- Although the gut microbiome is very stable in the long term, its specific composition **can fluctuate considerably in the short term.**



Changes in composition over time

How variable is the microbiome?

- **In the short term** (hours to days)
 - Changes after eating, defecation, change of environment, stress, use of medication (e.g. antibiotics)
- Fluctuations in the relative abundance of certain species
 - e.g. anaerobes such as *Bacteroides* or *Clostridium*

Article | [Open access](#) | Published: 18 November 2021

Temporal variability in quantitative human gut microbiome profiles and implications for clinical research

[Doris Vandeputte](#), [Lindsey De Commer](#), [Raul Y. Tito](#), [Gunter Kathagen](#), [João Sabino](#), [Séverine Vermeire](#),
[Karoline Faust](#) & [Jeroen Raes](#) 

[Nature Communications](#) **12**, Article number: 6740 (2021) | [Cite this article](#)

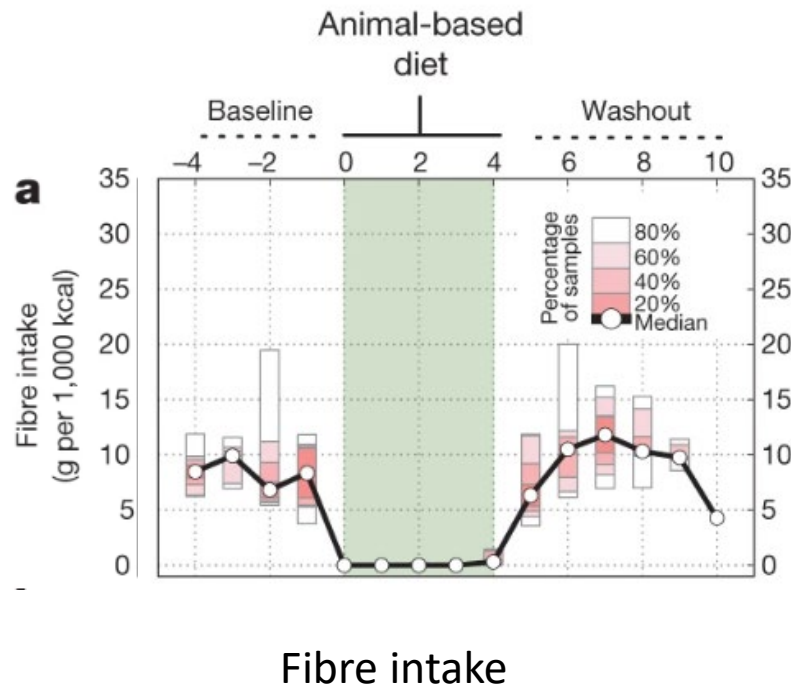
19k Accesses | 112 Citations | 145 Altmetric | [Metrics](#)

High variability – but the overall
composition quickly returns to its original
state – **the so-called resilience of the gut
microbiome ***

* Vandeputte et al. *Nat Commun.* 2021

How variable is the microbiome?

- **Short term** (hours to days)
 - Dietary intervention (n=10): high-fat diet (animal-based)



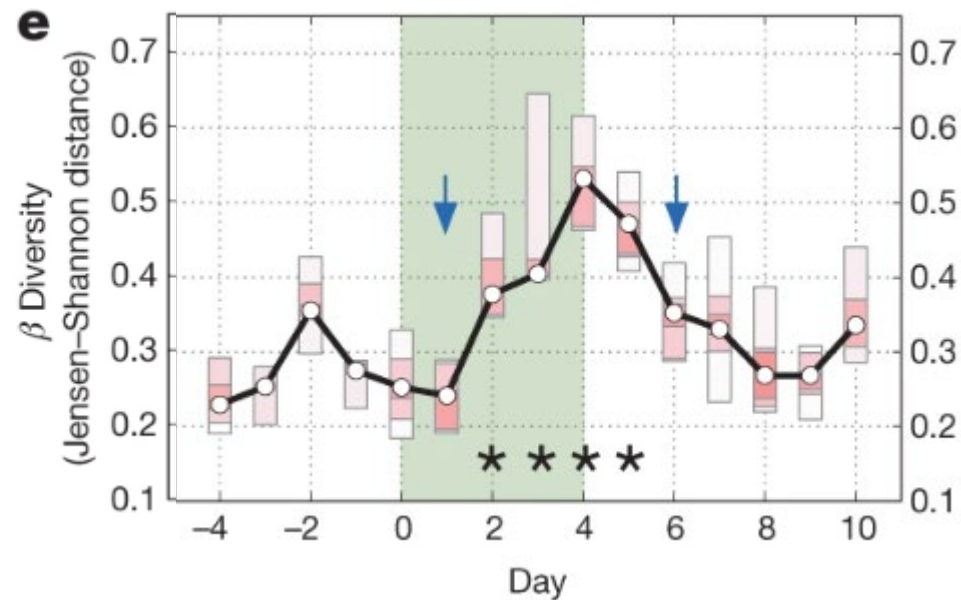
Letter | Published: 11 December 2013

Diet rapidly and reproducibly alters the human gut microbiome

[Lawrence A. David](#), [Corinne F. Maurice](#), [Rachel N. Carmody](#), [David B. Gootenberg](#), [Julie E. Button](#), [Benjamin E. Wolfe](#), [Alisha V. Ling](#), [A. Sloan Devlin](#), [Yug Varma](#), [Michael A. Fischbach](#), [Sudha B. Biddinger](#), [Rachel J. Dutton](#) & [Peter J. Turnbaugh](#)

[Nature](#) 505, 559–563 (2014) | [Cite this article](#)

198k Accesses | 1953 Altmetric | [Metrics](#)



Dramatic changes within 48 hours with extreme dietary change, but return to original state within another 48 hours

Factors influencing variability

- **Long term** (months to years)
 - In adults, the microbiome tends to be relatively stable unless there are major interventions (e.g., antibiotics, FMT).
 - In childhood and old age, the microbiome is generally more dynamic and less stable
 - Stability is supported by a consistent lifestyle, diet, and absence of antibiotics

Factor	Effect on variability
Antibiotics	Very strong, often causing disruption for months
Diet	moderate to significant (especially with extreme changes)
Travel	temporary changes (e.g. traveller's diarrhoea)
Age	The microbiome of children and seniors is more variable
Chronic diseases	e.g. IBD or metabolic syndrome alter stability

Freely adapted from Vandeputte et al. *Nat Commun.* 2021

What are the problems IV.

Potentially incorrect interpretation of microbiome health/dysfunction

- The host-microbiome relationship is **highly individual**, and the (un)beneficial nature of a particular microbiome composition always depends on the current context.
- With the exception of clear pathogens, **it is impossible to say with certainty which specific bacteria are beneficial to a given host and which are not.**

Notable exceptions (it seems): *Faecalibacterium prausnitzii* and a protozoan called *Blastocystis*

What are the problems V.

Offer of personalised food supplements

The current state of scientific knowledge and our technical capabilities **do not** currently **allow us** to predict the exact "behaviour" of the microbiome or, based on an analysis of its composition, **to prepare an individualised probiotic or prebiotic preparation** that will lead to a predefined, specific, long-term and sustainable change.

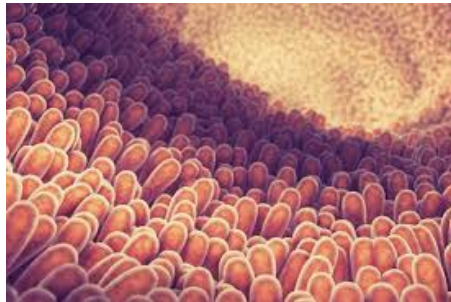
SUMMARY

- Commercial analyses (so far):
 - **They cannot provide targeted advice**
 - **Cannot** be used to prepare individualised meal plans or tailor-made probiotics
 - **It is therefore important to consider carefully whether it is worth spending so much money on them**

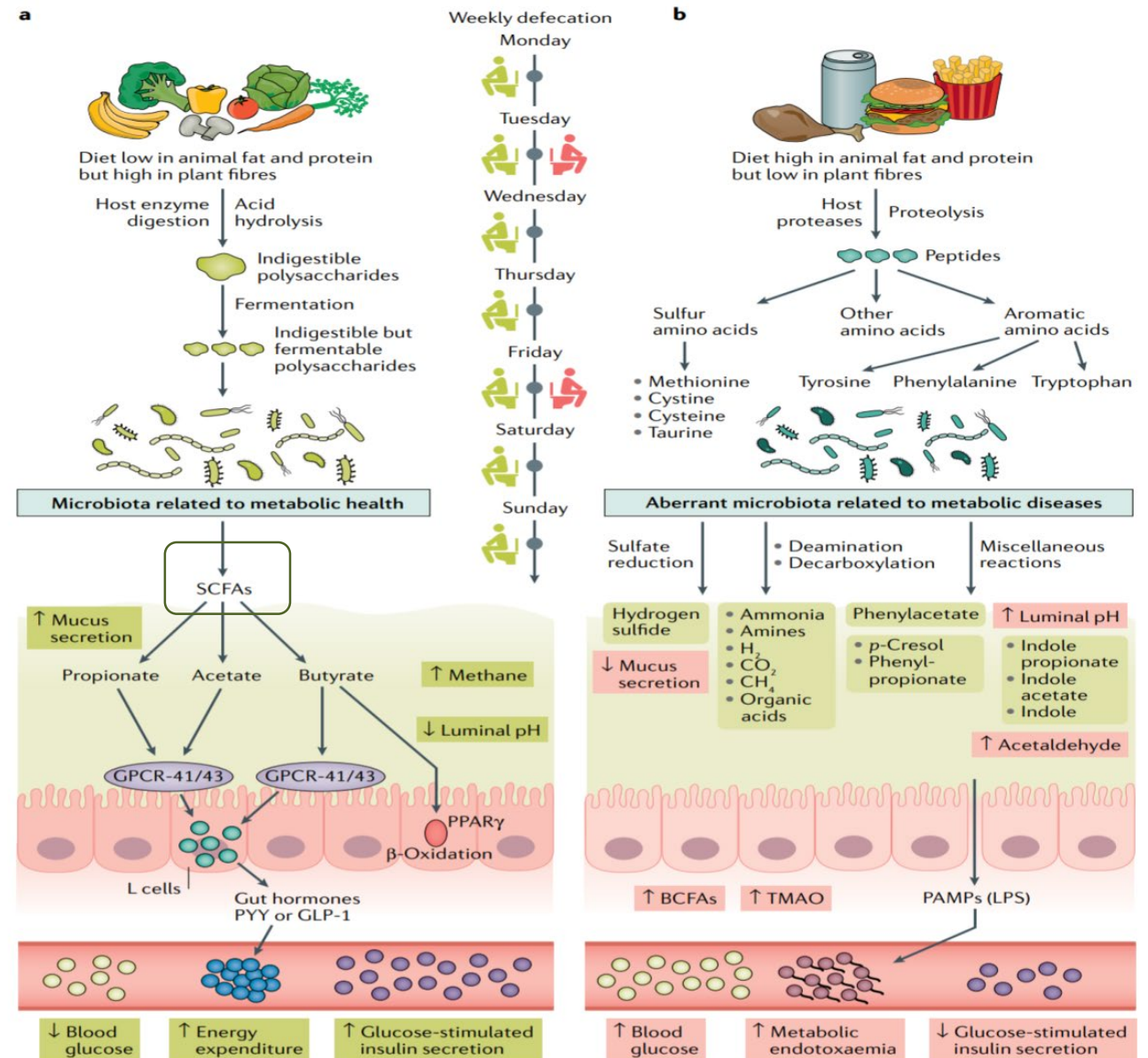
How to take care of your microbiome

Fibre

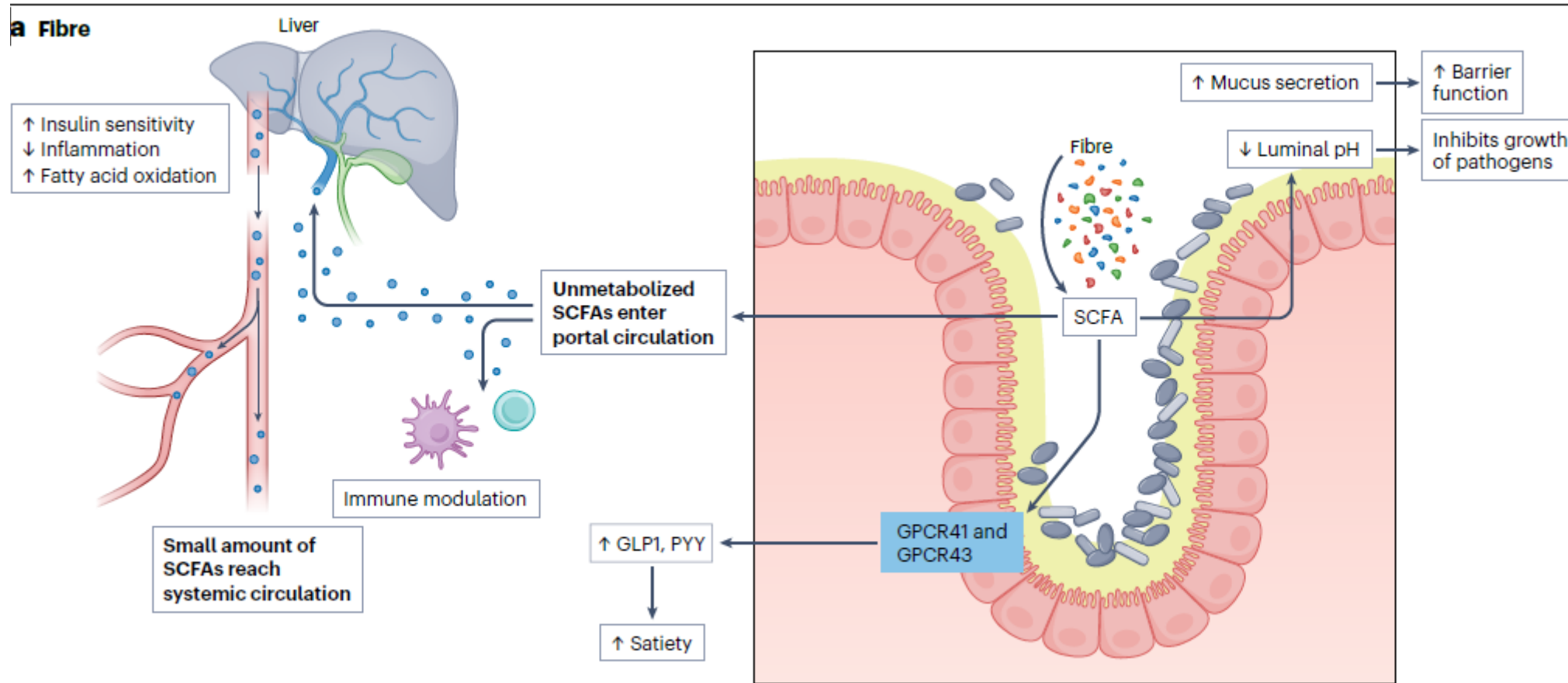
Adequate fibre intake (especially soluble fibre) **promotes SCFA production**



Supports intestinal epithelial function

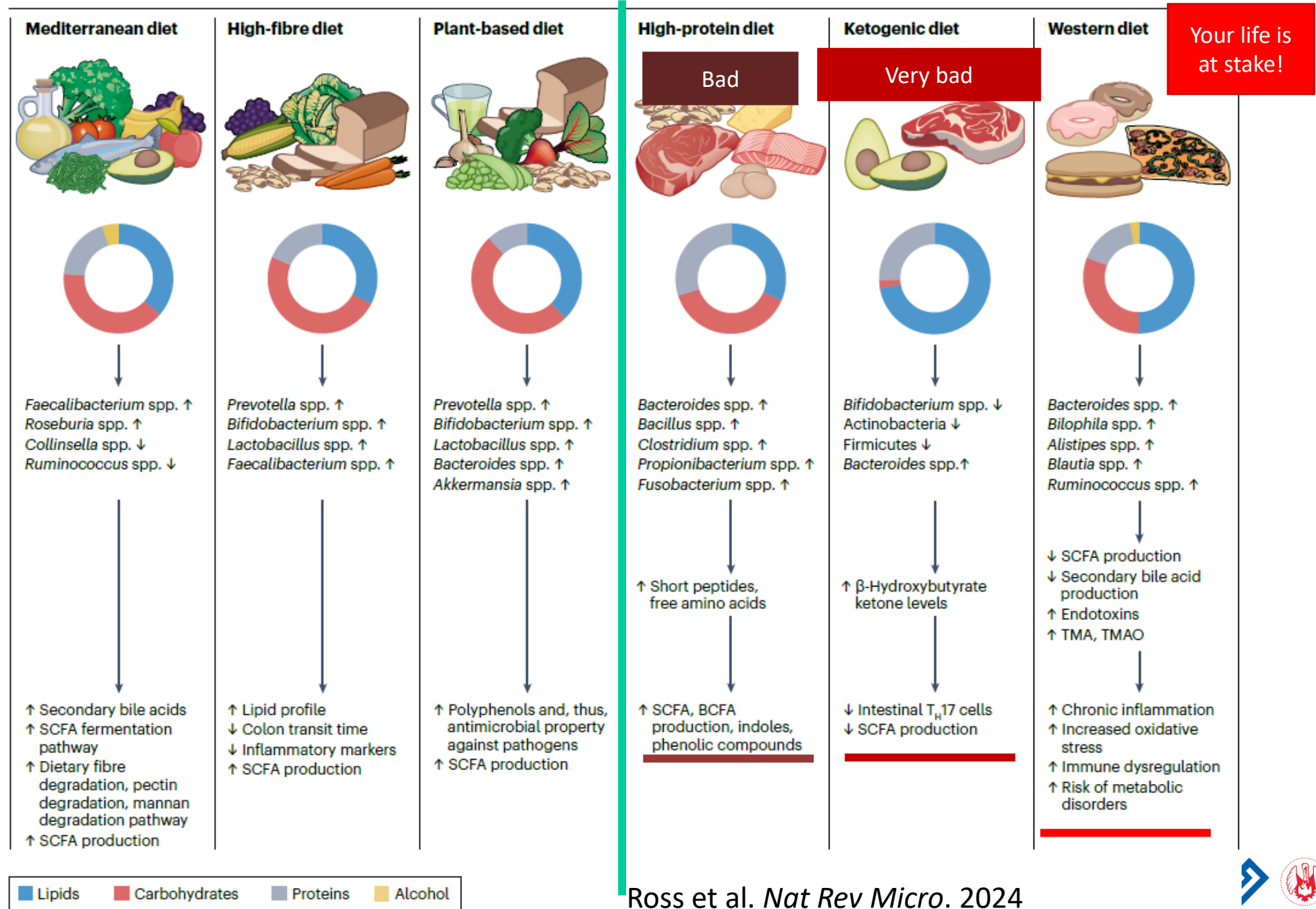


And not just food for the intestinal epithelium. It increases sensitivity to INS, reduces the production of pro-inflammatory molecules and increases MK oxidation



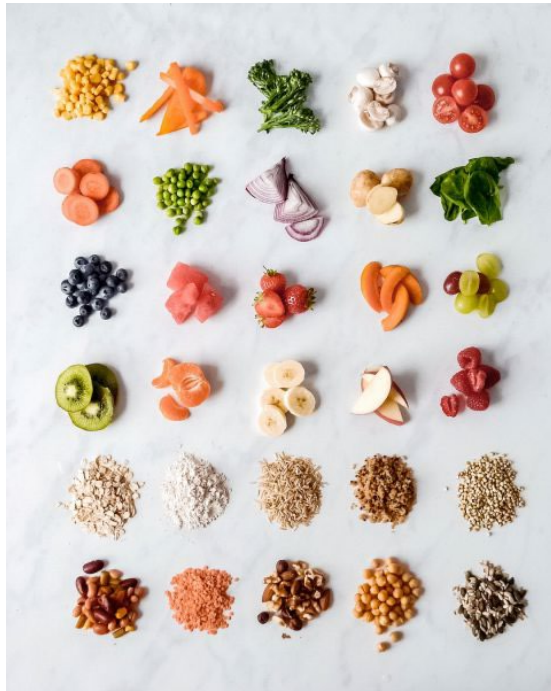
Ross et al. *Nat Rev Micro.* 2024

We are what we eat



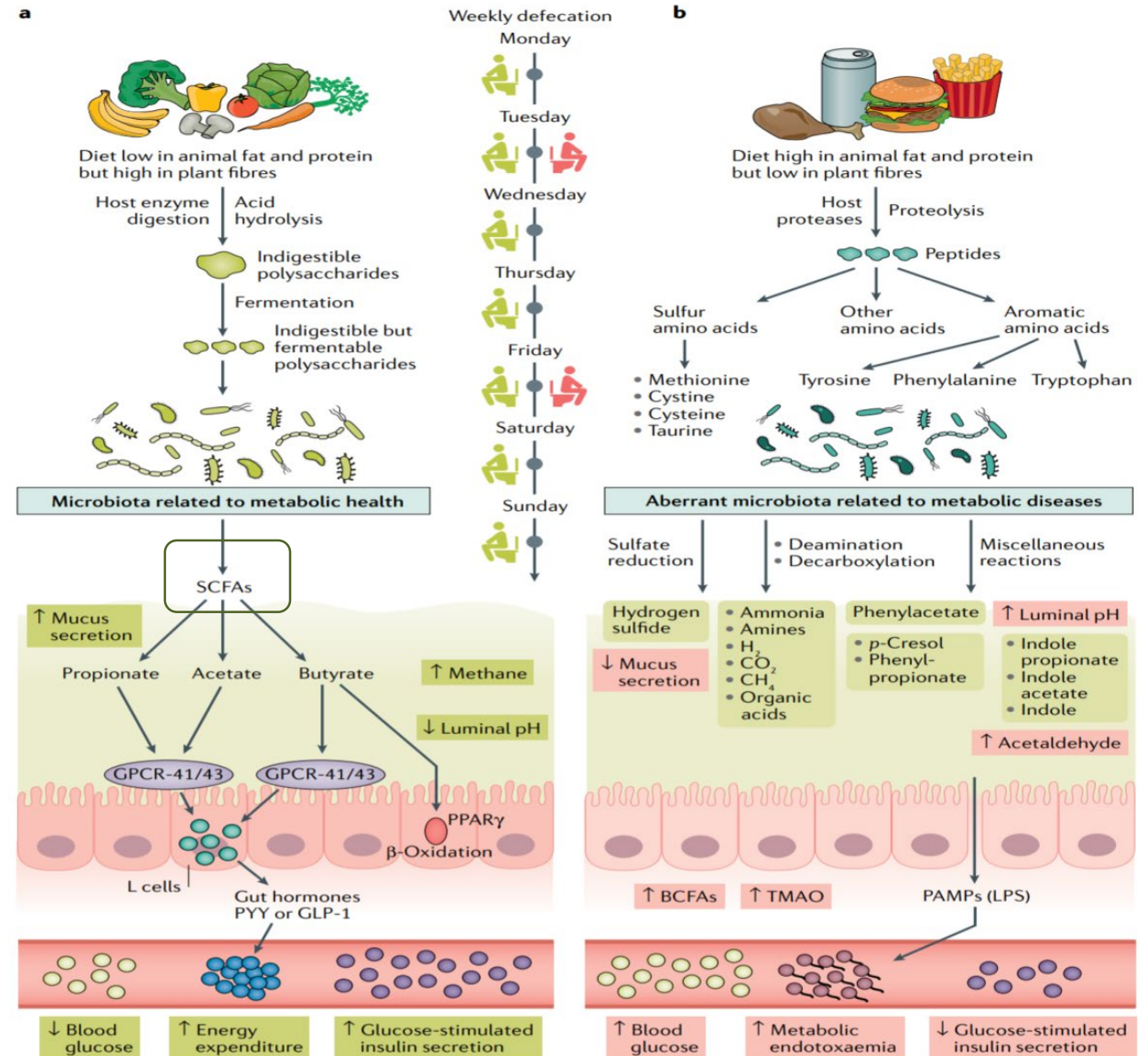
Ross et al. *Nat Rev Micro.* 2024

Confirmation of what was already known...



"Thirty different plants per week"

(Knight et al, American Gut Project, 2012)



Fan & Pedersen; *Nature Reviews Microbiology*, 2020

Practical recommendations

- **≥30 different plant-based foods per week** ^{1,2}
 - Regularly three servings of fermented foods per day (different types) ³
 - Main source of protein: less meat, more legumes and whole grain sources ^{1,2}
 - Limit ultra-processed foods that promote "bad" bacteria ²
- **Diversity > uniformity** – no single food or bacterium is a panacea; a balanced and varied diet is key

1) Knight et al, American Gut Project, 2012.

2) Fackelman et al. Nat Microbiology. 2024

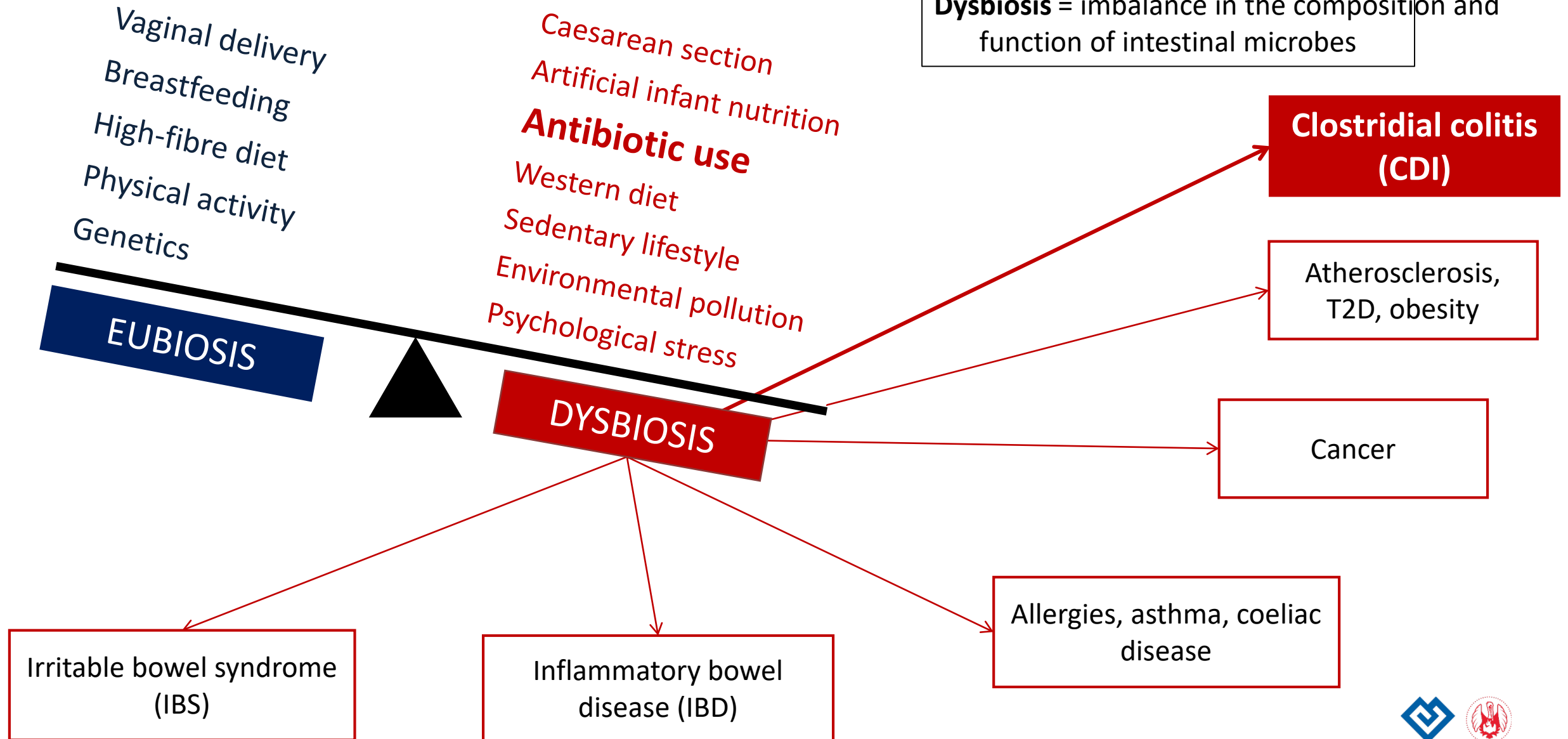
3) Asnicar et al. 2021. Cell

ATBs and their role in dysbiosis

The dysbiosis model and the effect of ATB treatment on the gut microbiome

Intestinal microbiome dysbiosis

Dysbiosis = imbalance in the composition and function of intestinal microbes



Difficilogenic ATB

- ATBs whose use is associated with a higher risk of **clostridial colitis**

4 "C"

Štefan, Antibiotics, 2024

Clindamycin
Cephalosporins
Co-aminopenicillins
Ciprofloxacin (+other FQs)

Open Forum Infectious Diseases

MAJOR ARTICLE

IDSA
Infectious Diseases Society of America

hivma
hiv medicine association

OXFORD

Comparison of Different Antibiotics and the Risk for Community-Associated *Clostridioides difficile* Infection: A Case–Control Study

Aaron C. Miller,^{1,2} Alan T. Arakkal,² Daniel K. Sewell,² Alberto M. Segre,³ Joseph Tholany,^{1,2} and Philip M. Polgreen,¹ CDC MinD-Healthcare Group

¹University of Iowa, Carver College of Medicine, Iowa City, Iowa, USA, ²University of Iowa, College of Public Health, Iowa City, Iowa, USA, and ³Department of Computer Science, University of Iowa, Iowa City, Iowa, USA

FMT outside the main indications

THE ONLY APPROVED INDICATION IS STILL
rCDI

Not this!



<https://www.youtube.com/watch?v=VMFSwMvac8I>

Overview of studies on FMT in direct treatment for non-CDI diagnoses		
Diagnosis	Number of RCTs	Summary of FMT use
Ulcerative colitis	13	Potential for inducing remission in ulcerative colitis; further research needed for long-term results
ARB carriage	1	Single RCT with non-significant conclusion, cohort studies show potential for decolonisation.
Crohn's disease	2	Promising results in Crohn's disease; stronger evidence needed for wider application.
Irritable bowel syndrome (IBS)	9	Some suggest improvement in IBS symptoms, but results are mixed; larger studies needed.
Metabolic syndrome	4	Evidence of effect on metabolic parameters (insulin sensitivity, lipids); requires combination with other interventions.
Hepatic encephalopathy	3	Results suggest that FMT may reduce the frequency of hepatic encephalopathy recurrence and improve patients' quality of life by modulating the gut microbiota and reducing systemic inflammation.
Chronic fatigue syndrome (CFS)	1	Possible effect on alleviating CFS symptoms by restoring the balance of the gut microbiota. However, the results are limited so far and further studies are needed.
Autism	0	Observational studies, some of which suggest alleviation of autism symptoms and improvement in gastrointestinal problems. However, no randomised controlled trials have been conducted to date, limiting the evidence to support this therapy for ASD.
Parkinson's disease	2	Promising initial results in influencing GI symptoms and possibly neurological outcomes; need for confirmation.
Obesity	3	Some studies show an effect on weight and metabolic health in obese patients. Results are mixed, with limited effect on some metabolic parameters.

Statement on FMT

- FMT may be accompanied by mild side effects (diarrhoea, cramps or abdominal pain, fever, bloating, flatulence and/or constipation), but some can be very serious or even fatal (however, these are usually the result of inadequate donor screening and the subsequent transplantation of pathogenic microorganisms).
- **FMT must therefore always be performed under strict medical supervision.** The Czech Medical Association strongly warns against unprofessional attempts at home FMT – this could endanger the health and even the life of the recipient! This is due to the need for thorough screening for the presence of pathogenic microorganisms in donated stool and the prevention of serious intestinal infections. Not to mention injuries resulting from self-administration or contamination during processing at home.

SUMMARY

- Irrational use of antibiotics leads to dysbiosis and thus to the risk of other diseases, not only CDI
- The most difficult antibiotic is clindamycin, but cephalosporins, potentiated aminopenicillins and fluoroquinolones are also high-risk.
- FMT is only approved for the treatment of recurrent *C. difficile* infection.

Is it the real key to health or
a commercial illusion?

What is its real impact on health and what are the limits of current knowledge?

- From a microbiome perspective, the saying "**we are what we eat**" applies.
 - **Limits:** is it the cause or the effect? We do not know.
- **Variability over time:** Need to monitor dynamics, not test once.
- **Resistance:** Antibiotics have a negative effect (but perhaps not permanently).

Can nutritional recommendations be personalised based on microbiome analysis?

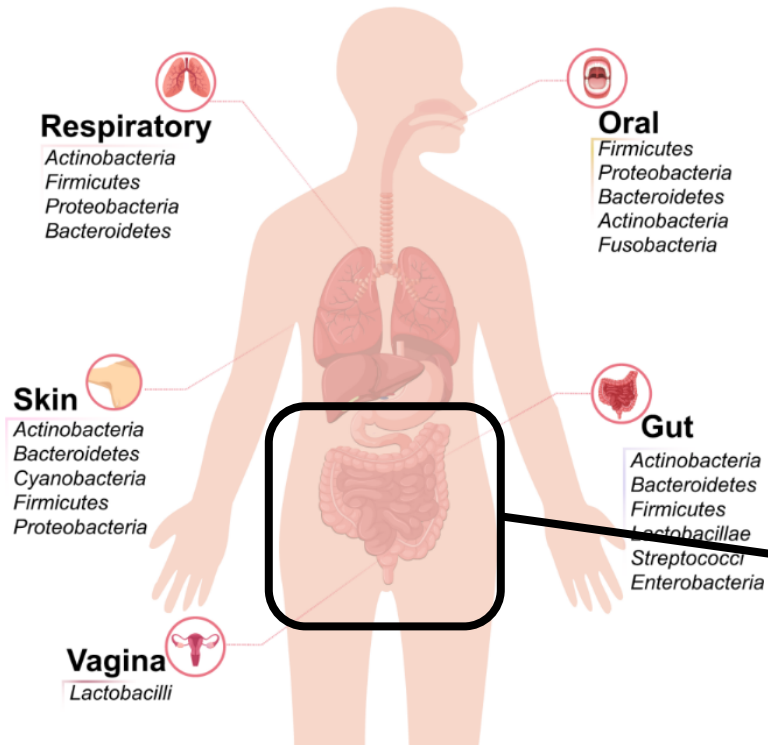
- **Not yet.** We can see what the microbiome characteristics of healthier people are.
- The recommendations are still the same and relatively simple – "**eat more than 30 plant-based foods a day**" (and these are not tailor-made diets or probiotics).

Physiological microbiota

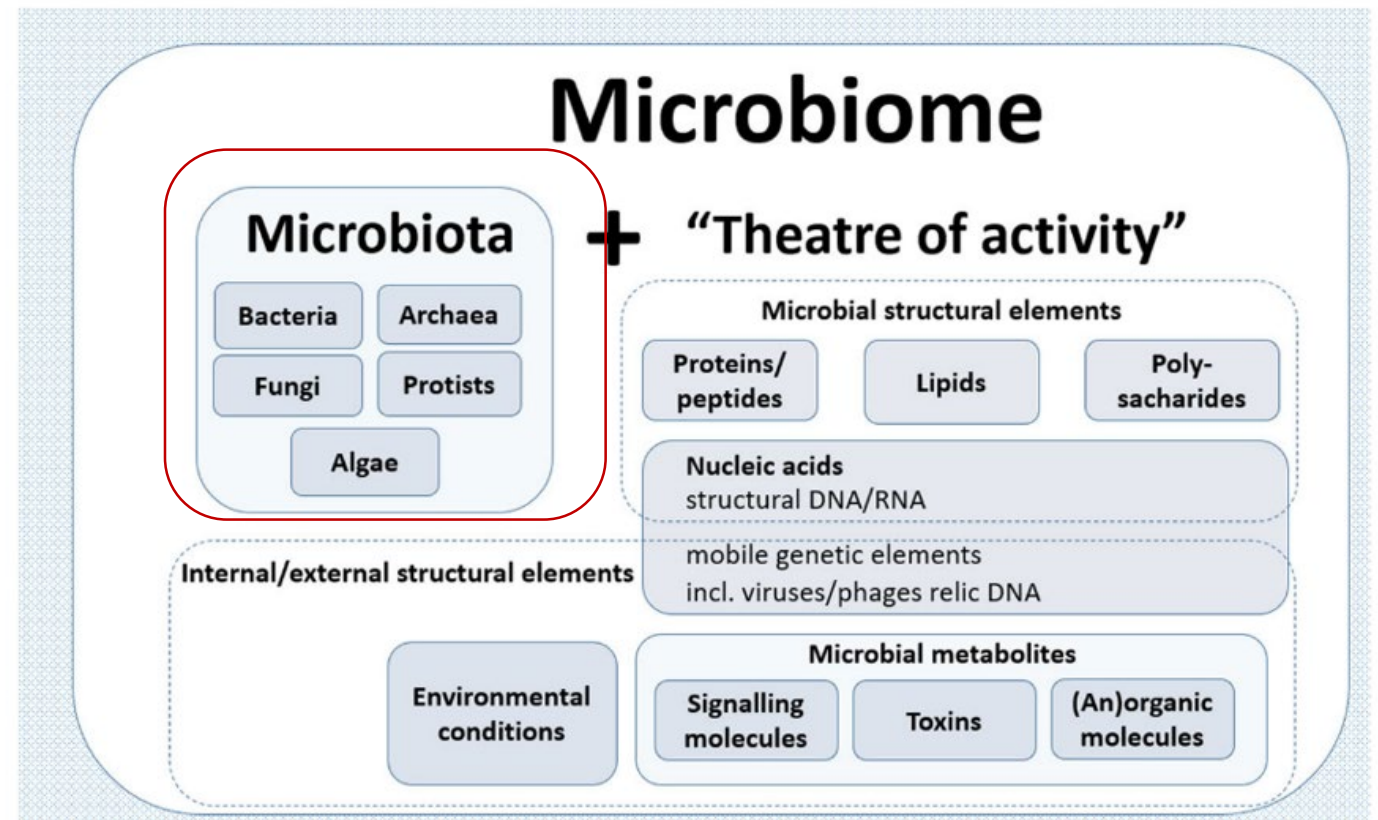
Microbiome

Microbiome = a characteristic microbial community that inhabits a specific, rationally defined habitat with typical physical and chemical conditions

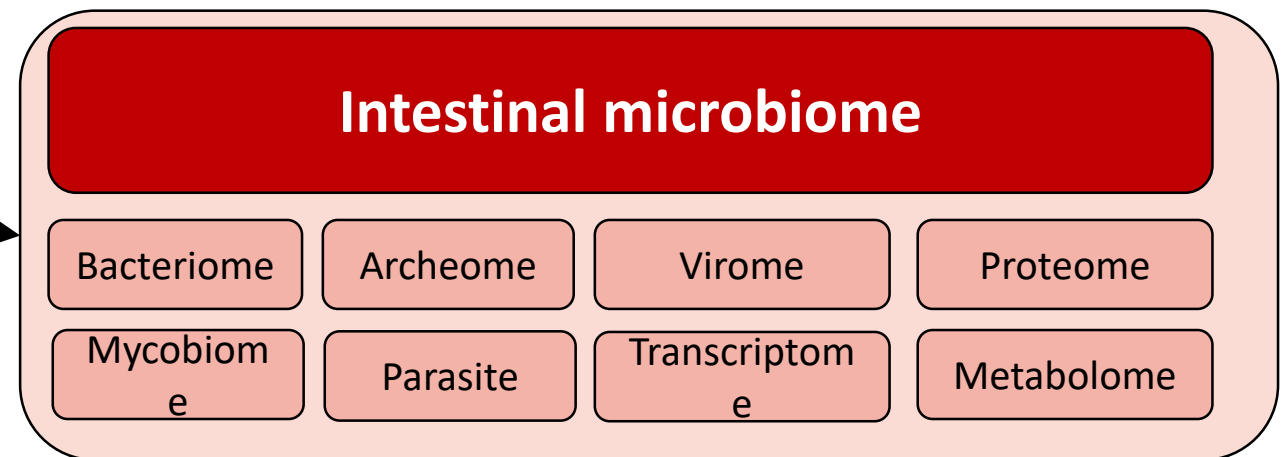
Berg et al, *Microbiome*, 2020



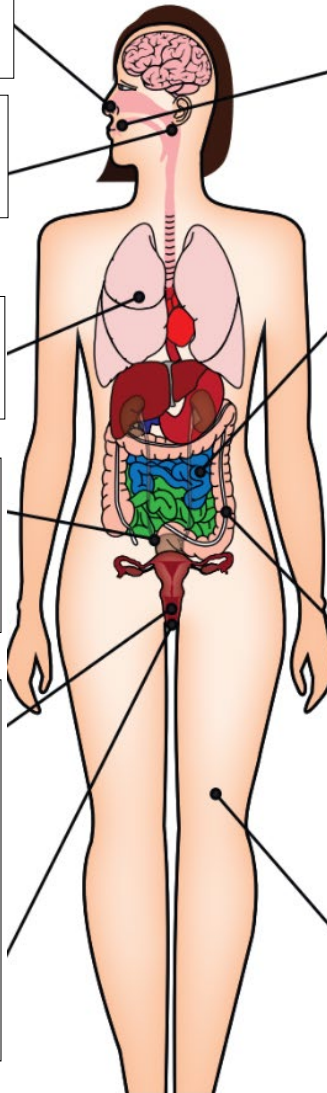
Hou et al, *Sig Transduct Target Ther*, 2022



Berg et al, *Microbiome*, 2020



PHYSIOLOGICAL MICROBIOTA



Coagulase-negative staphylococci, diphtheroids

Viridans streptococci, oral neisseria, diphtheroids

Viridans streptococci, oral neisseria, diphtheroids

Viridial streptococci (swallowed)

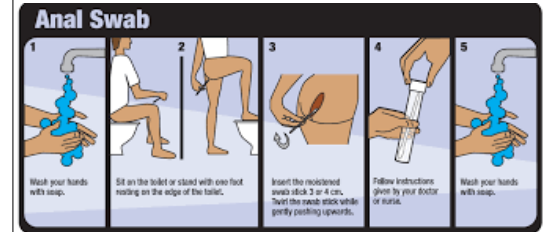
CoN staphylococci, diphtheroids, enterococci (10^3 and less in urine)

Lactobacilli, diphtheroids CoN staphylococci, viridial streptococci

Almost everything:

- except GI infections (Campylobacter, Salmonella, Yersinia)
- Toxigenic *C. difficile* and *H. pylori* (in stool)
- Parasites (*Cryptosporidium*, *Entamoeba histolytica* etc.) but not *Blastocystis* unless symptoms are present

Coagulase-negative staphylococci, diphtheroids, micrococci (*Cutibacterium acnes*)

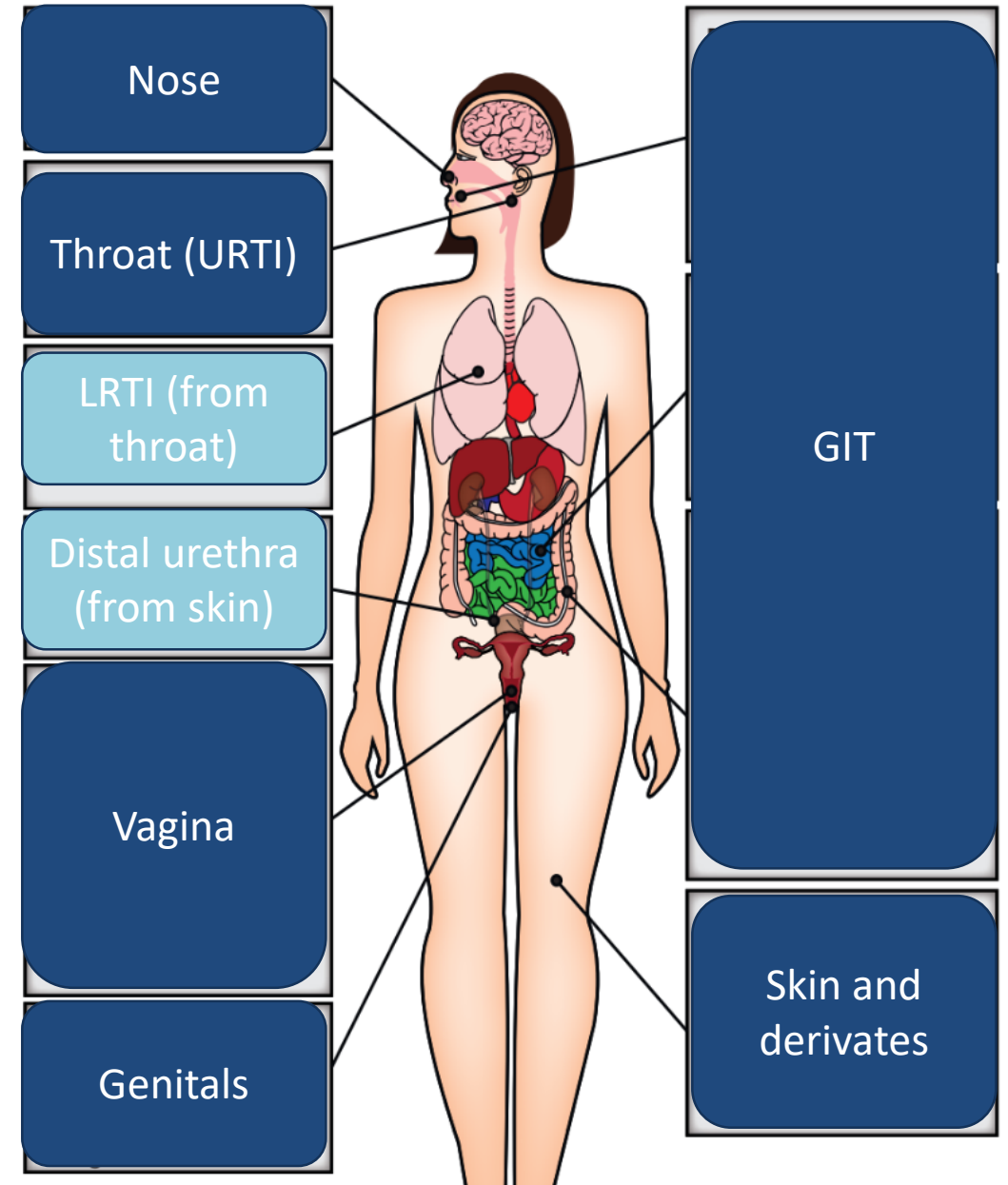


PHYSIOLOGICAL MICROBIOTA

Most common materials with physical microbiota

- **Skin swabs**
- Nasal and nasopharyngeal swabs
- Throat swab
- Vaginal swab
- Rectal and stool swabs

(Sputum and aspirates from DCD – but this is contamination from HCD)



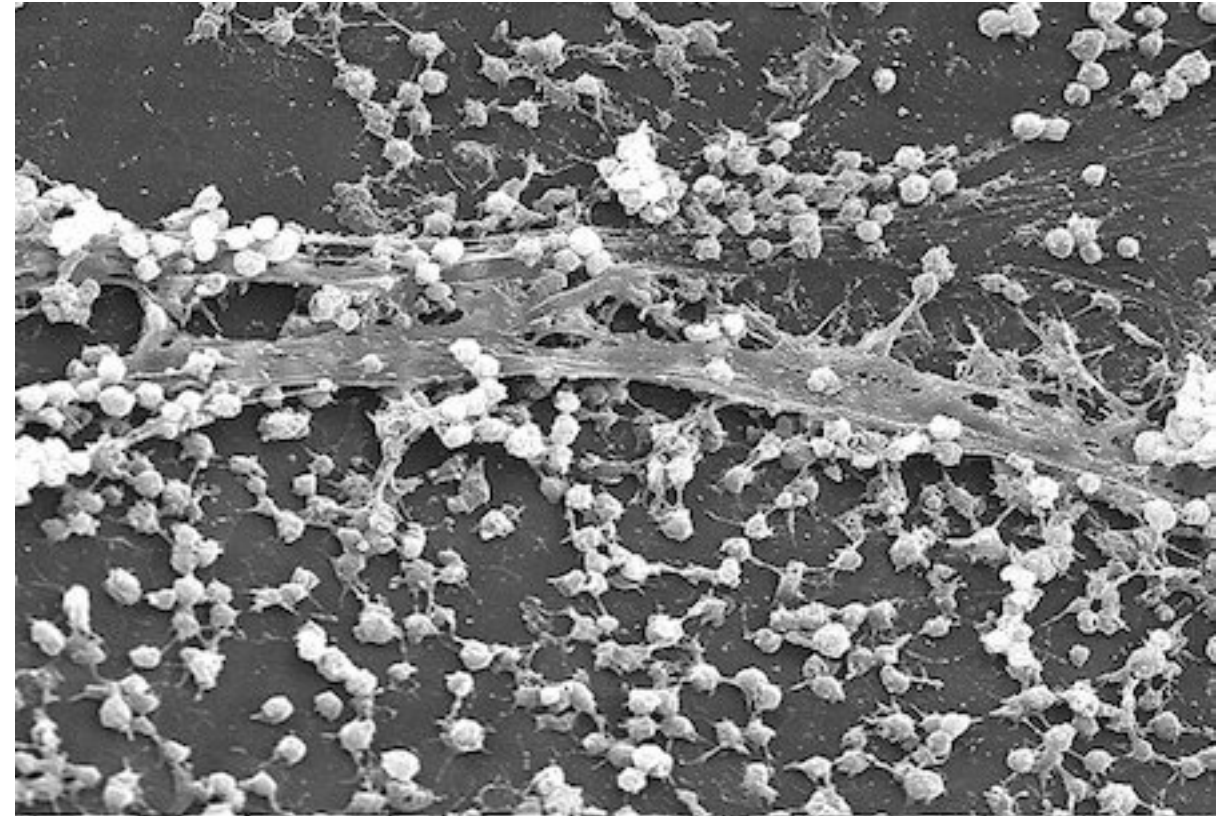
Material	Physiological findings
Skin swab	Coagulase-negative staphylococci, diphtheroids
Nasal and nasopharyngeal swabs	Coagulase-negative staphylococci, diphtheroids, <i>S. aureus</i> carriage
Throat swab	Viridans streptococci and Neisseria, anaerobes
Sputum and aspirates from DCD	Almost "sterile"
Vaginal swab	Lactobacilli, coagulase-negative staphylococci
Rectal swab and stool	Enterobacteria, enterococci, coagulase-negative staphylococci

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Name	Haemolysis on KA	Pathogenicity	
<i>S. aureus</i>	Yes	+++	Physiologically in the nose (approx. 20%); Pathogenic potential: - Soft tissues, orthopaedic, pneumonia (! PVL+), UTI, BSI, - Enterotoxigenesis, STSS, SSSS
CoNS are not just <i>S. epidermidis</i> and <i>S. saprophyticus</i>			
<i>S. capitis</i>	Yes	+	Physiologically on the skin; Colonisation of catheters, prostheses and valves
<i>S. epidermidis</i>	No	+	
<i>S. hominis</i>	No	+	
<i>S. haemolyticus</i>	Yes	+	
<i>S. lugdunensis</i>	Yes	++	Physiologically on the skin; Soft tissues, orthopaedics, endocarditis, BSI
<i>S. saprophyticus</i>	No	++	Physiologically on the skin; UTI

Coagulase-negative staphylococci

- Where does it make sense to address this? In all materials with a risk of **biofilm** formation
 - Blood cultures*
 - Catheters with significant quantities*
 - Orthopaedic materials (tissues, swabs, punctures)
 - Wound swabs from spinal surgery patients
 - Deep wounds with signs of infection



Staphylococcus aureus biofilm collected from an infected indwelling catheter (*The Role of Bacterial Biofilms in Antimicrobial Resistance*, ASM, 2023)

BEWARE OF THESE FINDINGS with physiological microbiota

Everything in a sterile sample:

- ☐ Tissues
- ☐ Heart valves
- ☐ Blood cultures (except for one of several bottles, where CoNS – suspected contamination)
- ☐ Joint aspirate
- ☐ CSF

Among others:

- ☐ More than 10^3 CFU from suprapubic puncture
- ☐ STDs pathogens in children
- ☐ *E. coli* from stool sample in children under 2 years of age

THANK YOU FOR YOUR ATTENTION

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