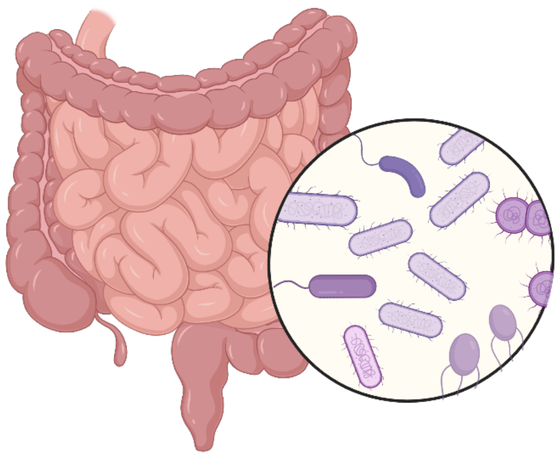
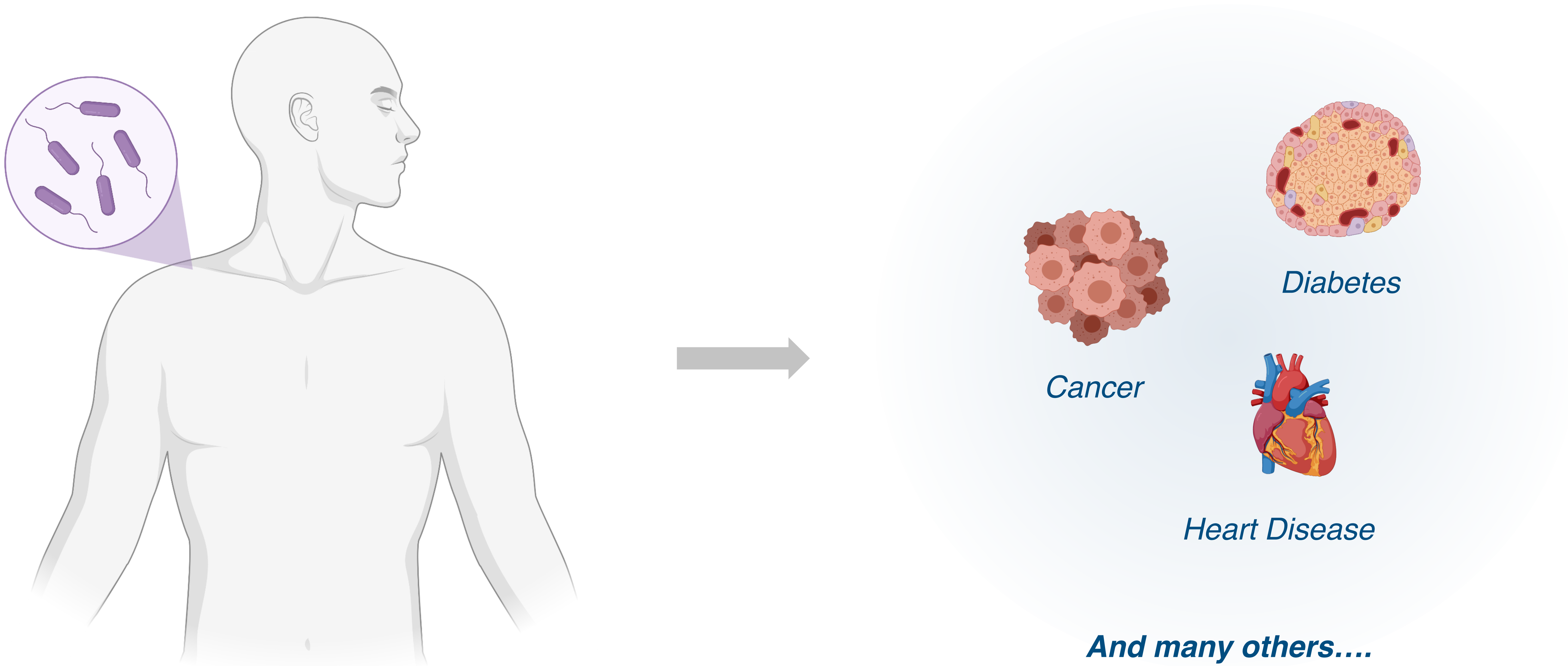


Gut Microbiota in Human Metabolic Health and Disease

Olivia Garry
MacMillan Group Meeting
March 16th, 2021

Gut Microbiome is Important for Human Health

Considerable part of the environmental influence on human health and disease risk ***may be mediated or modified by microbial communities***



Lynch, S. V.; Perdersen, O. *N. Engl. J. Med.* **2016**, *375*, 2369

Outline

Overview of Gut Microbiome

Microbial Metabolites and Metabolic Health

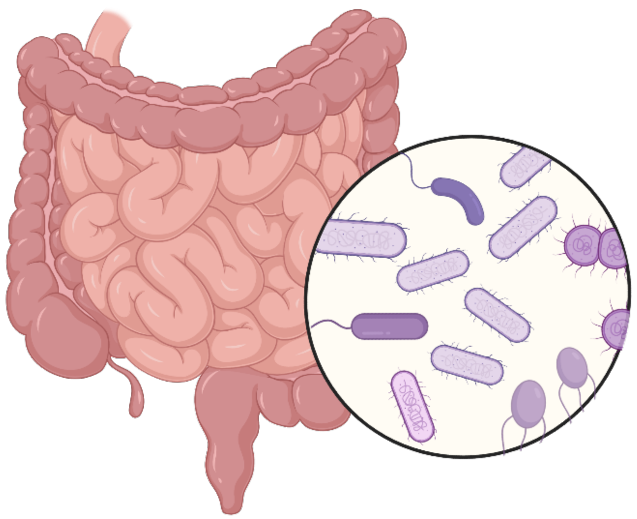
- BCAAs
- Imidazole Propionate
- SCFAs

Gut Microbiome and Metabolic Disease

- Obesity
- Cardiovascular Disease

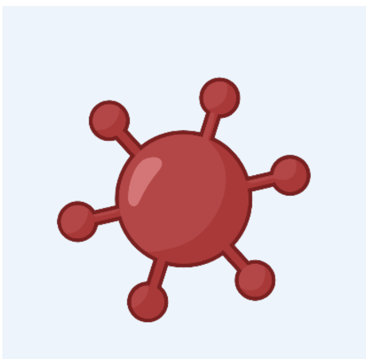
Interventions

- Diet
- Drugs and Pre/Pro/Postbiotics
- Bioengineered Commensals
- Fecal Microbiota Transplantation

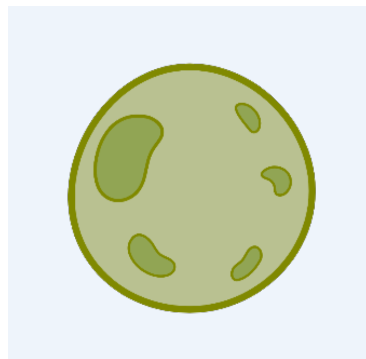


Microbe vs. Microbiome vs. Microbiota

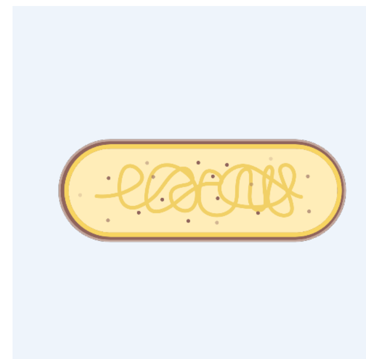
Microbe (or microorganism) = Organism which is too small to be seen by the naked eye



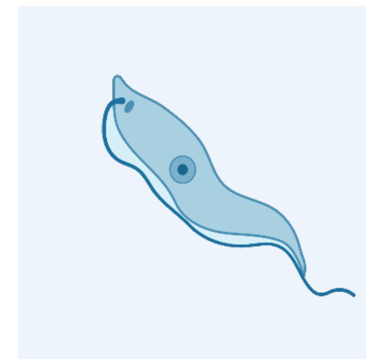
Viruses



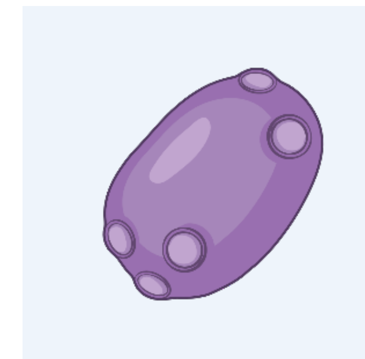
Algae



Bacteria

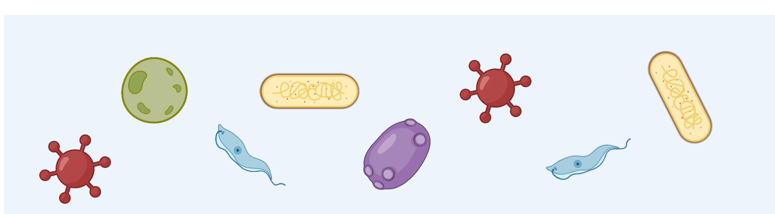


Protozoa



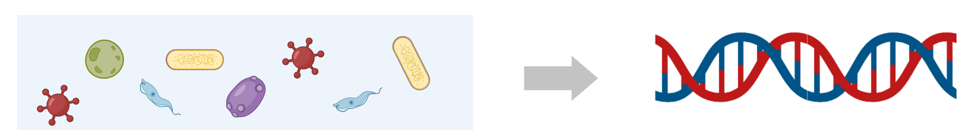
Fungi

Microbiota



The microorganisms found within a specific environment

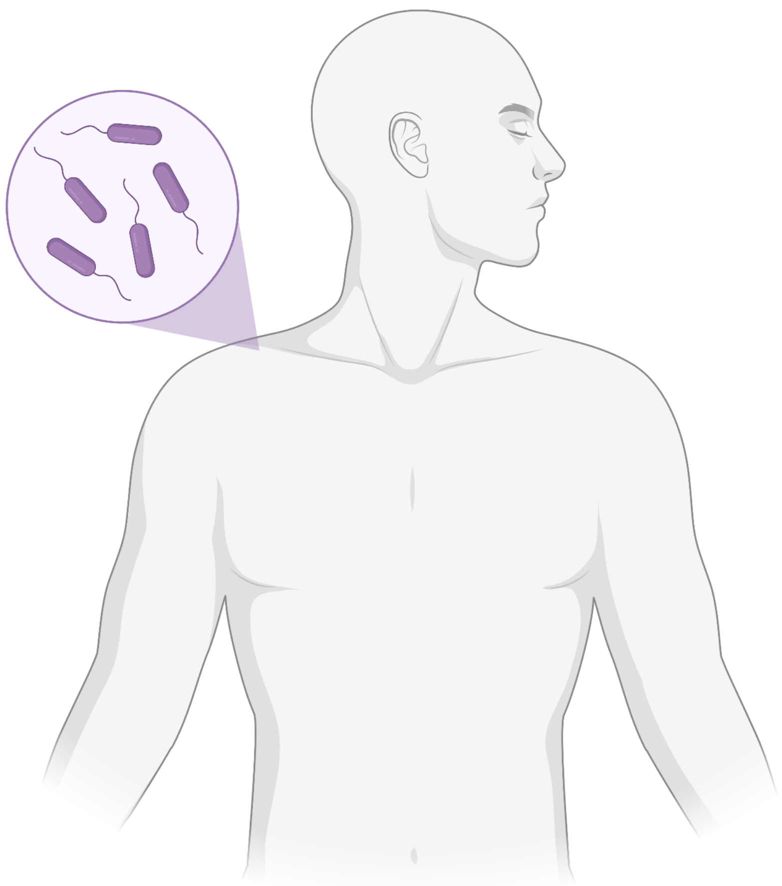
Microbiome



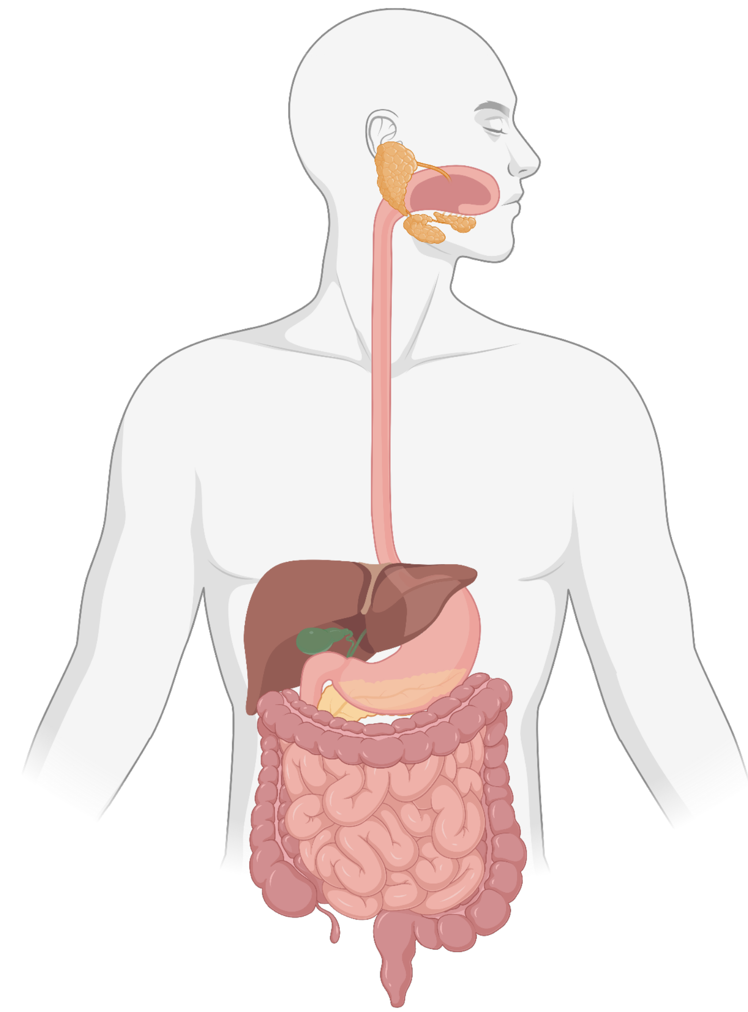
Collection of genomes from all microorganisms in an environment

Pedersen, O.; Fan, Y. *Nat. Rev. Microbiol.* **2021**, *19*, 55

Gut Microbiota



vast majority of our microbes reside in the gut



Human Microbiota

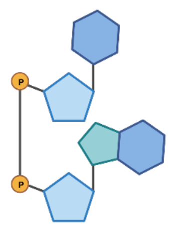
Microbes on a human comprise about 1–3% of body mass

Gut Microbiota

Microorganisms found in the gut (mouth to anus)

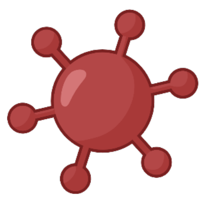
Pedersen, O.; Fan, Y. *Nat. Rev. Microbiol.* **2021**, *19*, 55

What does the Gut Microbiota do?



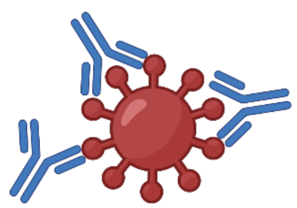
Aids Metabolism

e.g digestion, synthesis of essential vitamins



Protection

First line of defence against pathogens



Regulation of Immune *System*

e.g. via SCFAs

A healthy gut microbiome is essential for human health

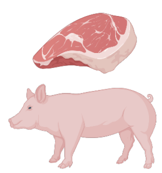
Germ-free (GF) animals (*no microbes*) have multiple abnormalities

Colonization with gut microbiota partially resolves abnormalities

Pedersen, O.; Fan, Y. *Nat. Rev. Microbiol.* **2021**, *19*, 55

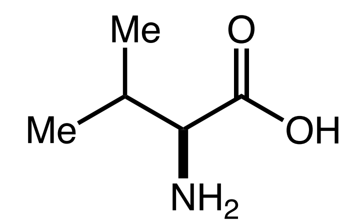
Gut Microbiota Metabolism

For their own energy supply, gut microbiota ferment energy-yielding nutrients



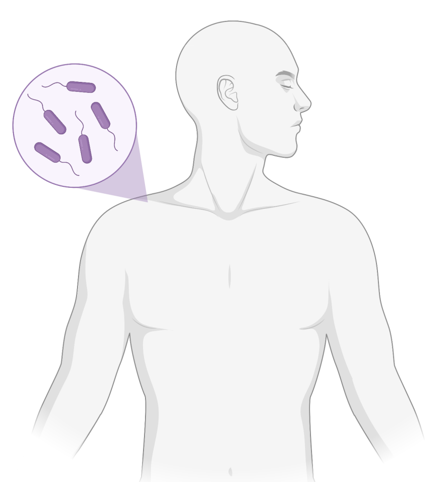
Food Source

Specific Bacteria



Metabolite

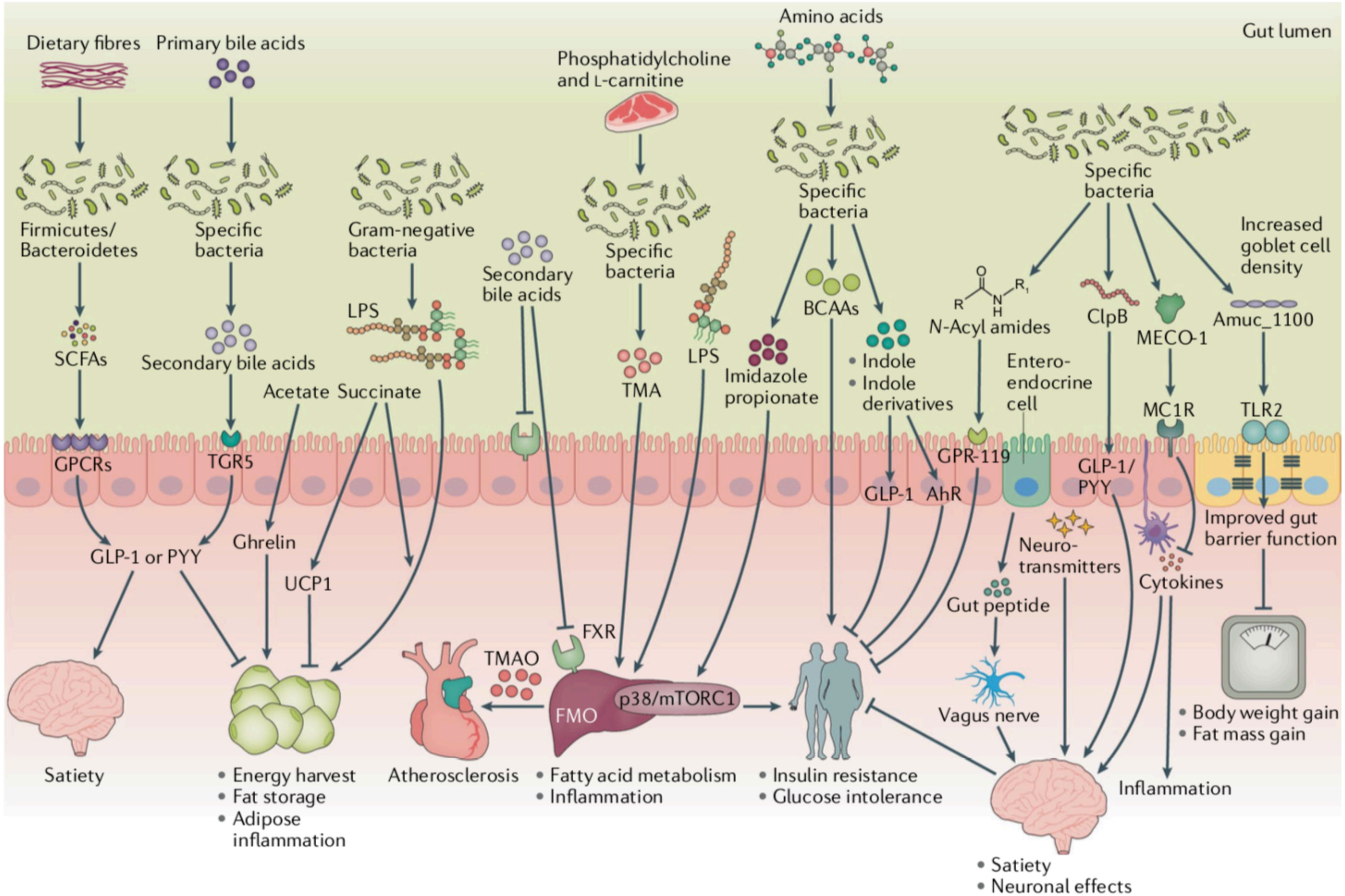
5–10% of energy needs come from these fermented nutrients



Metabolite can lead to biological effect in host

Pedersen, O.; Fan, Y. *Nat. Rev. Microbiol.* **2021**, *19*, 55

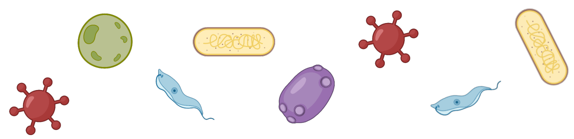
Gut Microbiota Metabolism



Key Takeaway: ***Gut microbiota metabolism leads to microbial metabolites which affect the host***

Pedersen, O.; Fan, Y. *Nat. Rev. Microbiol.* **2021**, *19*, 55

What does a Healthy Gut Microbiome look like?



High taxa diversity

(# and evenness of species)

High microbial gene richness

(number of unique genes)

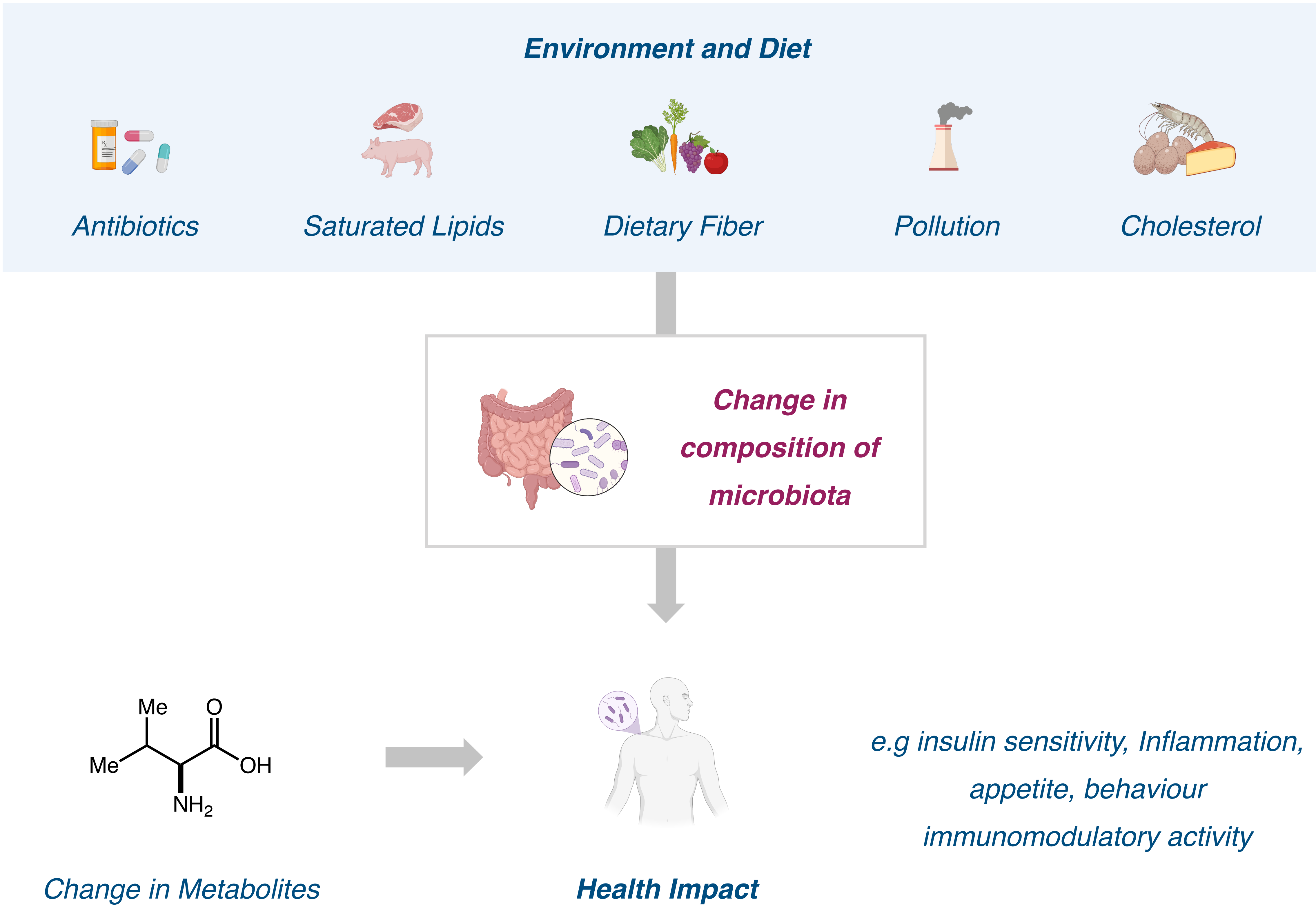
Stable microbiome functional cores

Genes encoding glycosaminoglycan degradation, production of SCFAs, biosynthesis of essential amino acids and vitamins

Huge variation between individuals - ***No golden standard***

Huttenhower, C. et al. *Nature* **2012**, *486*, 207

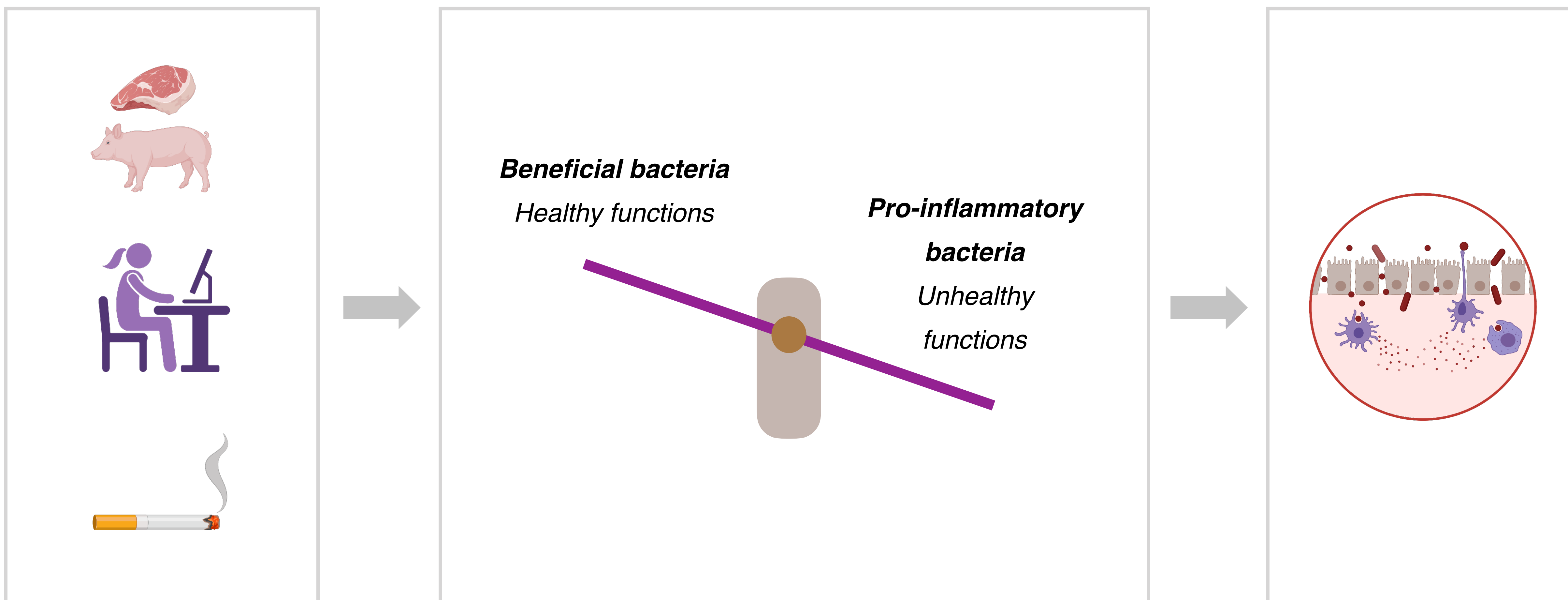
Environment Alters the Gut Microbiota



Pedersen, O.; Fan, Y. *Nat. Rev. Microbiol.* **2021**, *19*, 55

Gut Dysbiosis

Gut dysbiosis → Disturbance to gut microbiota homeostasis due to an ***imbalance in microbiota, changes in their metabolic activity or changes in their local distribution***

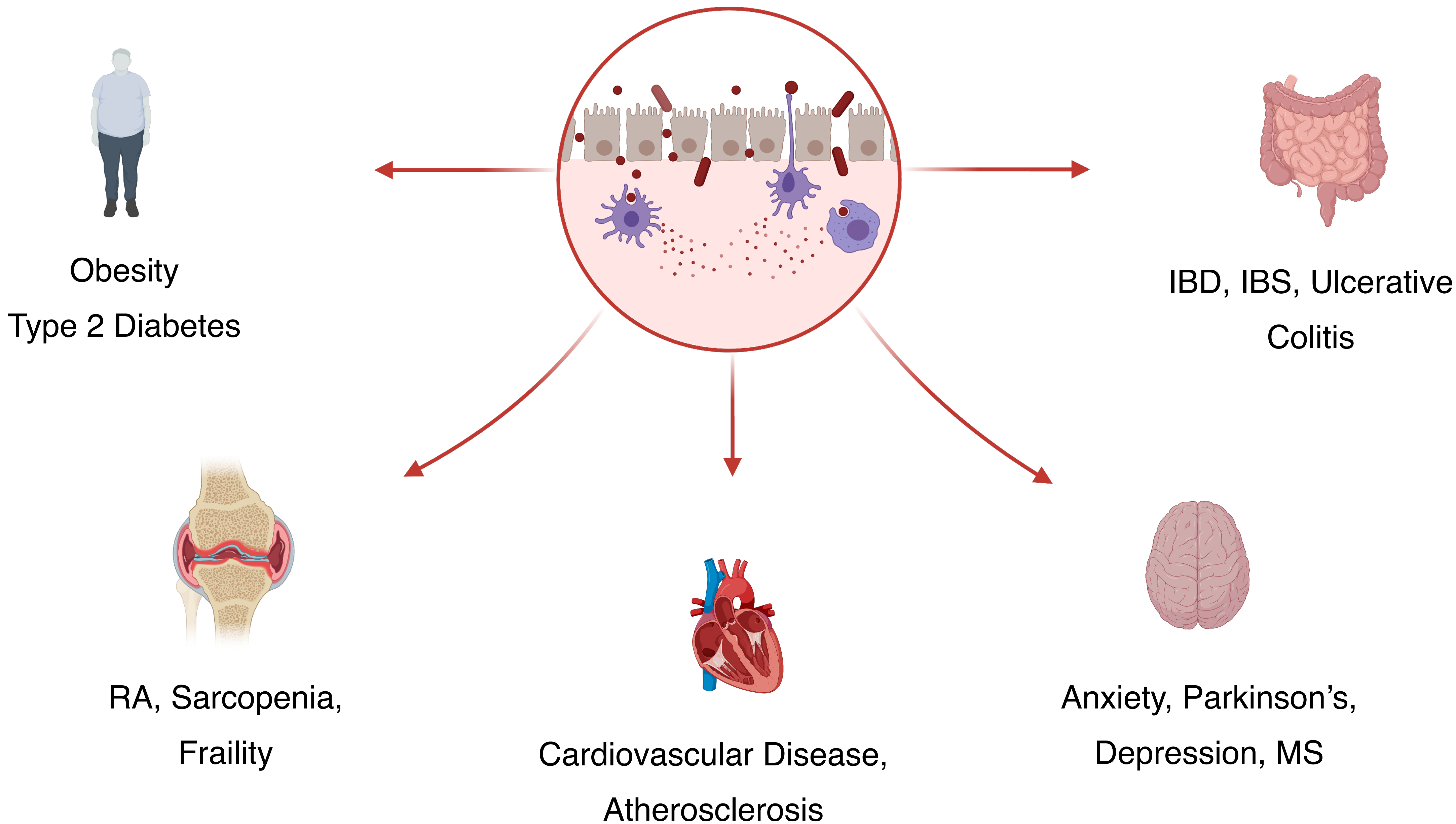


Trigger | ***Out-of-Balance Gut Microbiome*** | ***Gut Dysbiosis***

Pedersen, O.; Fan, Y. *Nat. Rev. Microbiol.* **2021**, *19*, 55

Gut Dysbiosis and Health and Disease

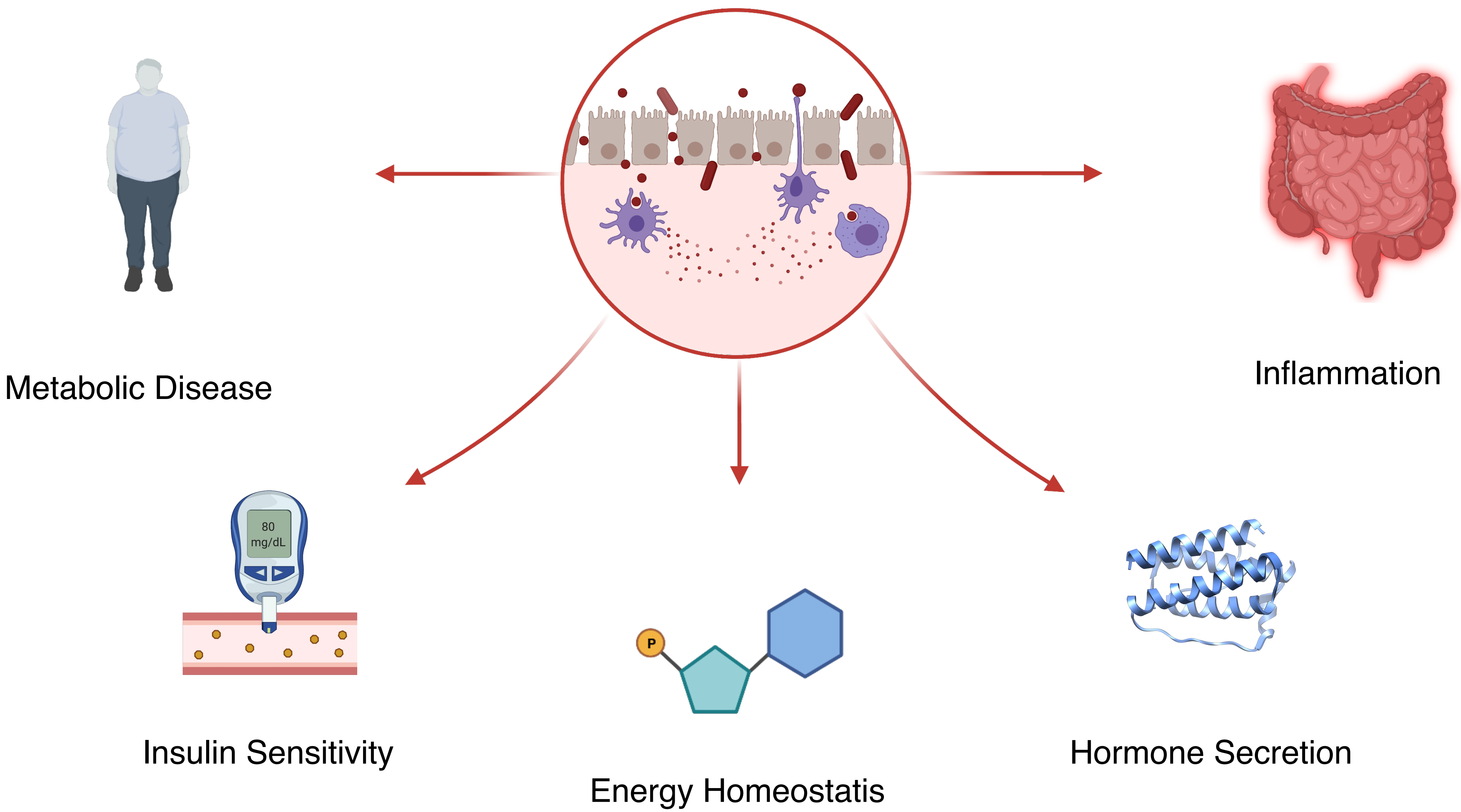
Gut dysbiosis has been associated with many diseases although causation proved for few of them



Baptista, L. C.; Sun, Y.; Carter, C. S.; Buford, T. W. *Front. Nutr.* **2020**, *7*, 709

Gut Dysbiosis and Metabolic Health

Metabolic Health → Have an overall metabolism that is linked to derivable life quality and longevity



Pedersen, O.; Fan, Y. *Nat. Rev. Microbiol.* **2021**, *19*, 55

Outline

Overview of Gut Microbiome

Microbial Metabolites and Metabolic Health

SCFAs

BCAAs

Imidazole Propionate

Gut Microbiome and Metabolic Disease

Obesity

Cardiovascular Disease

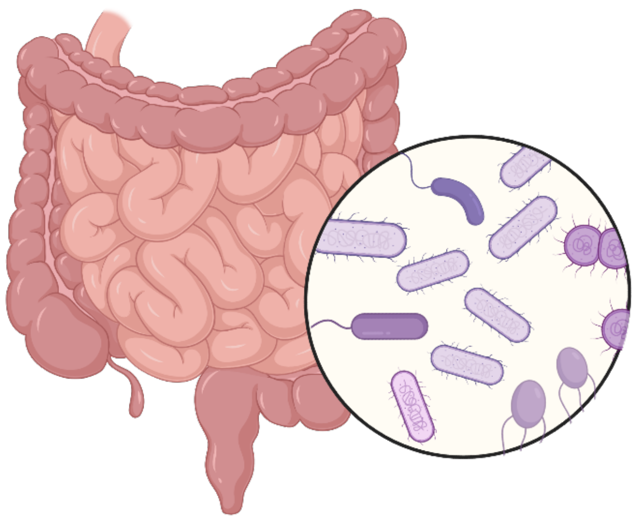
Interventions

Diet

Drugs and Pre/Pro/Postbiotics

Bioengineered Commensals

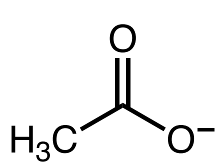
Fecal Microbiota Transplantation



Microbial Metabolites affect Metabolic Health

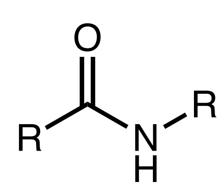
Intestinal microbial products affect host energy homeostasis, body adiposity, glucose tolerance, insulin sensitivity, inflammation and endocrine regulation

Dietary fibres → *Firmicutes/ Bacteroidetes* →



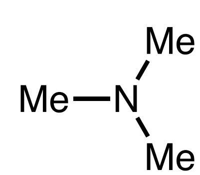
Short Chain Fatty Acids

- → *Specific bacteria* →



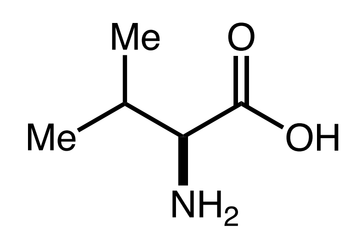
e.g LPS, N-acyl amides, ClpB, MECO-1

Phosphatidylcholine and L-carnitine → *Specific bacteria* →



TMA

Amino acids → *Specific bacteria* →



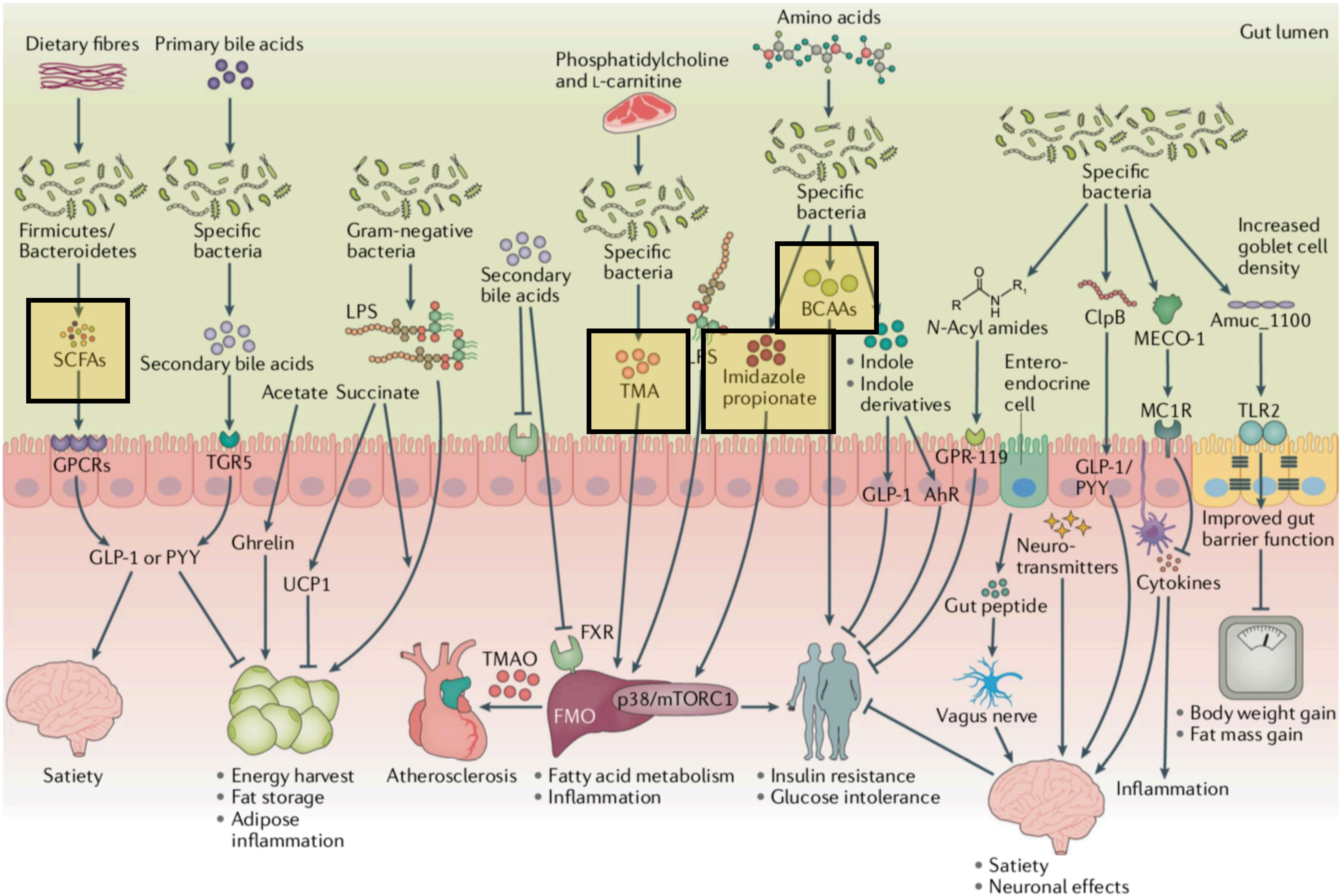
Branched Chain Amino Acids

Bile acids → *Specific bacteria* →

Secondary Bile Acids

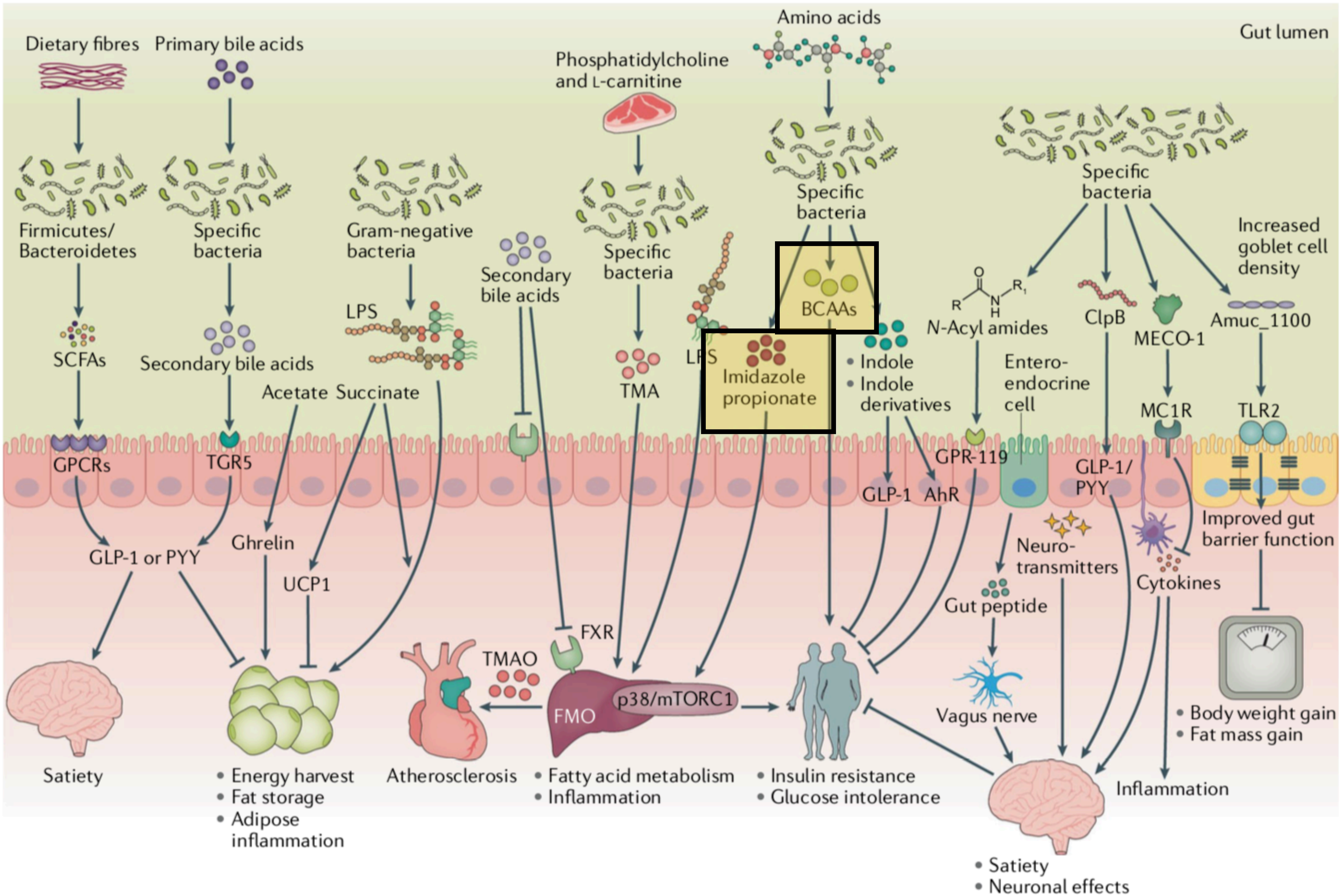
Pedersen, O.; Fan, Y. *Nat. Rev. Microbiol.* **2021**, *19*, 55

Microbial Metabolites affect Metabolic Health



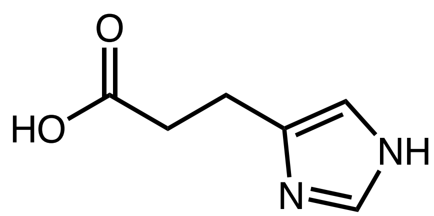
Pedersen, O.; Fan, Y. *Nat. Rev. Microbiol.* **2021**, *19*, 55

Microbial Metabolites affect Metabolic Health



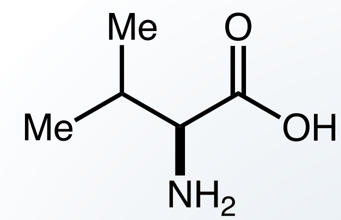
Pedersen, O.; Fan, Y. *Nat. Rev. Microbiol.* **2021**, *19*, 55

Amino Acid Derived Metabolites Linked in Insulin Resistance

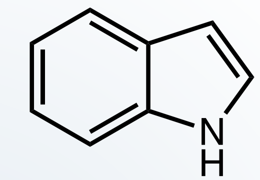


Amino acids
e.g histidine

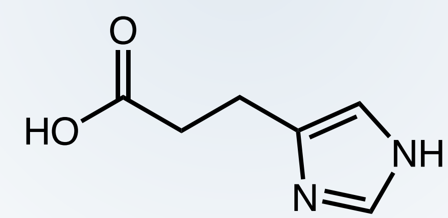
Specific Bacteria



BCAAs



Indole and derivatives



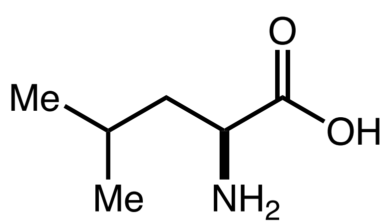
Imidazole Propionate

Amino acid-derived microbial metabolites play a role in insulin resistance

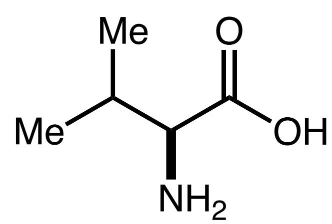
Pedersen, O.; Fan, Y. *Nat. Rev. Microbiol.* **2021**, *19*, 55

Case Study: BCAAs and Insulin Sensitivity

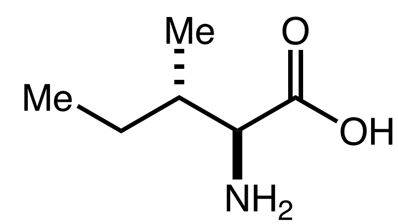
Elevated circulating concentration of the BCAAs ***leucine, isoleucine and valine are a strong biomarker for insulin resistance***



Leucine



Isoleucine



Valine

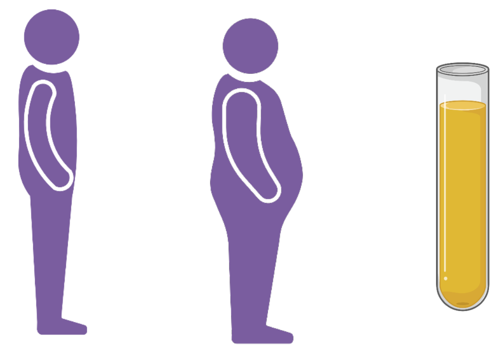
BCAA =

branched-chain amino acid

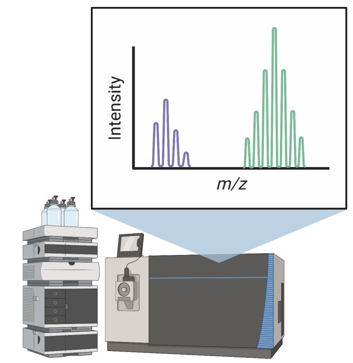
Model	Isoleucine	Leucine	Valine	Tyrosine	Phenylalanine	Isoleucine, tyrosine and phenylalanine
Models adjusting for age, sex, BMI and fasting glucose (*n* = 378)						
Metabolite as continuous variable						
Per s.d.	1.70 (1.27–2.28)	1.62 (1.20–2.17)	1.57 (1.17–2.09)	1.85 (1.35–2.55)	2.02 (1.40–2.92)	2.42 (1.66–3.54)
P	0.0004	0.001	0.002	0.0001	0.0002	<0.0001
Metabolite as categorical variable						
First quartile	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
Second quartile	1.11 (0.58–2.10)	2.40 (1.24–4.68)	1.49 (0.75–2.94)	1.89 (0.94–3.81)	1.39 (0.74–2.59)	3.48 (1.68–7.23)
Third quartile	2.14 (1.07–4.27)	3.15 (1.46–6.84)	2.15 (1.05–4.42)	3.26 (1.56–6.84)	2.12 (1.04–4.32)	2.82 (1.25–6.34)
Fourth quartile	3.14 (1.51–6.55)	3.66 (1.61–8.29)	3.14 (1.43–6.86)	2.82 (1.25–6.34)	2.28 (1.00–5.20)	5.99 (2.34–15.34)
P for trend	0.001	0.004	0.003	0.010	0.035	0.0009

Wang, T. J. et al. *Nat. Med.* **2011**, *17*, 448

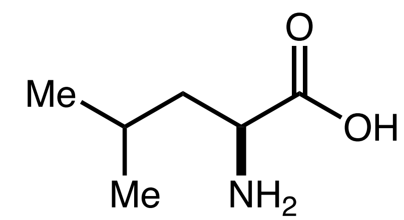
Case Study: BCAAs and Insulin Sensitivity



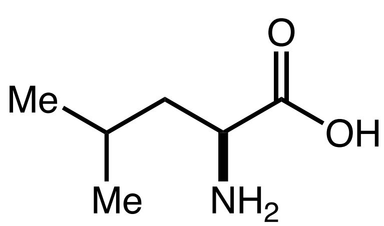
Serum of diseased and healthy patients

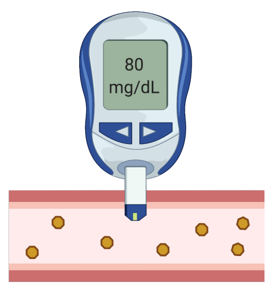


Mass spectrometry analysis



Metabolites (Metabolome)





Correlate with phenotypes

Untargeted metabolomics finds that ***BCAAs are associated with insulin resistance***

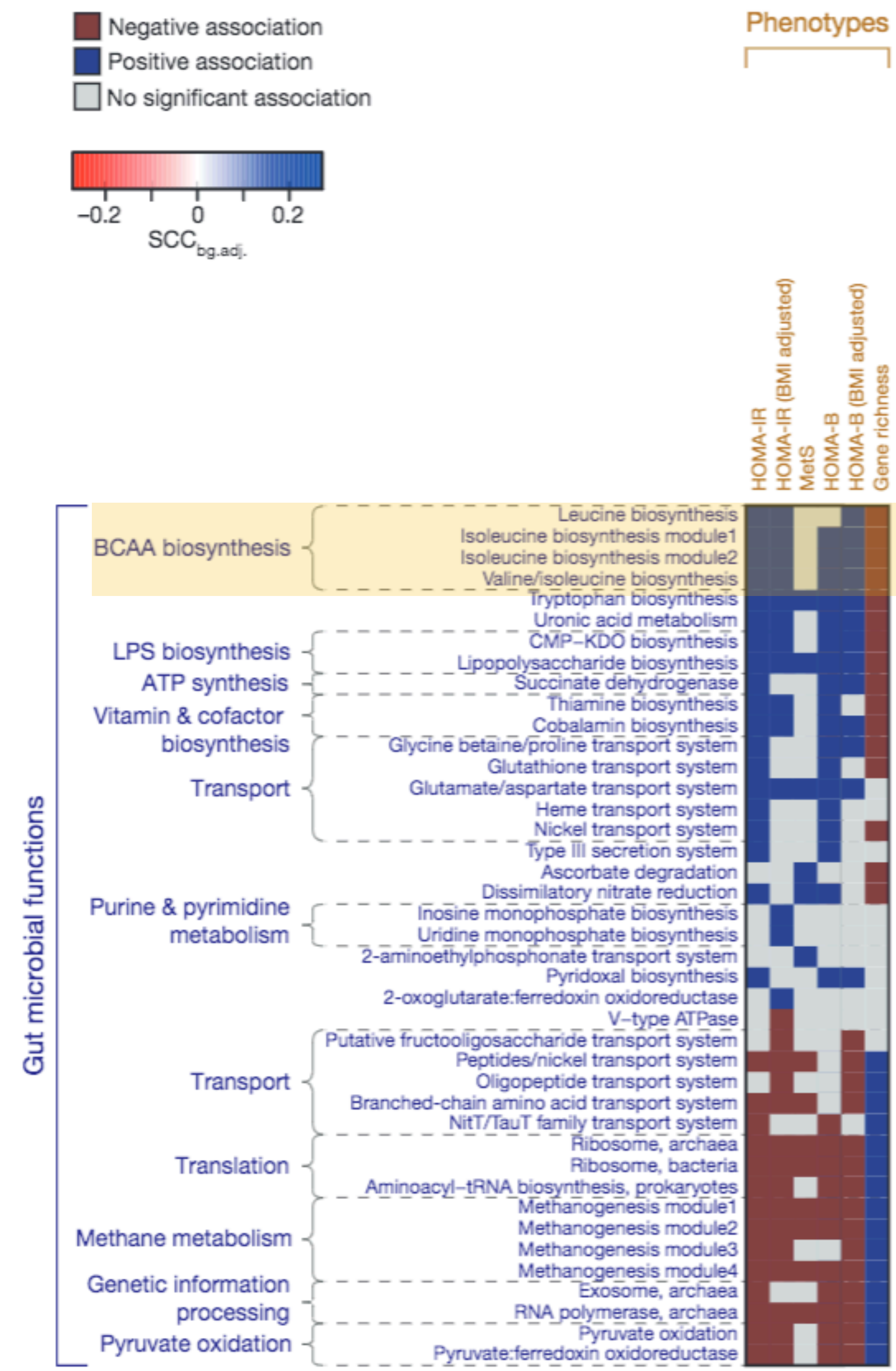
Pedersen, H. K. et al. *Nature*, **2016**, *535*, 376

Case Study: BCAAs and Insulin Sensitivity

Measure the microbiome of the patients

Plugged the gut microbiome (genes) into KEGG database to investigate metabolic potential of these metabolites

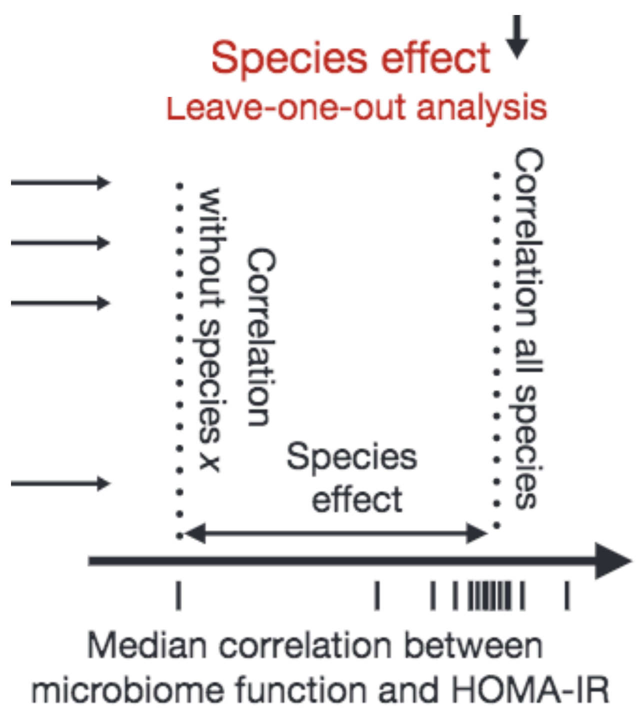
Functional modules correlated with insulin resistance include enzymes for BCAA biosynthesis

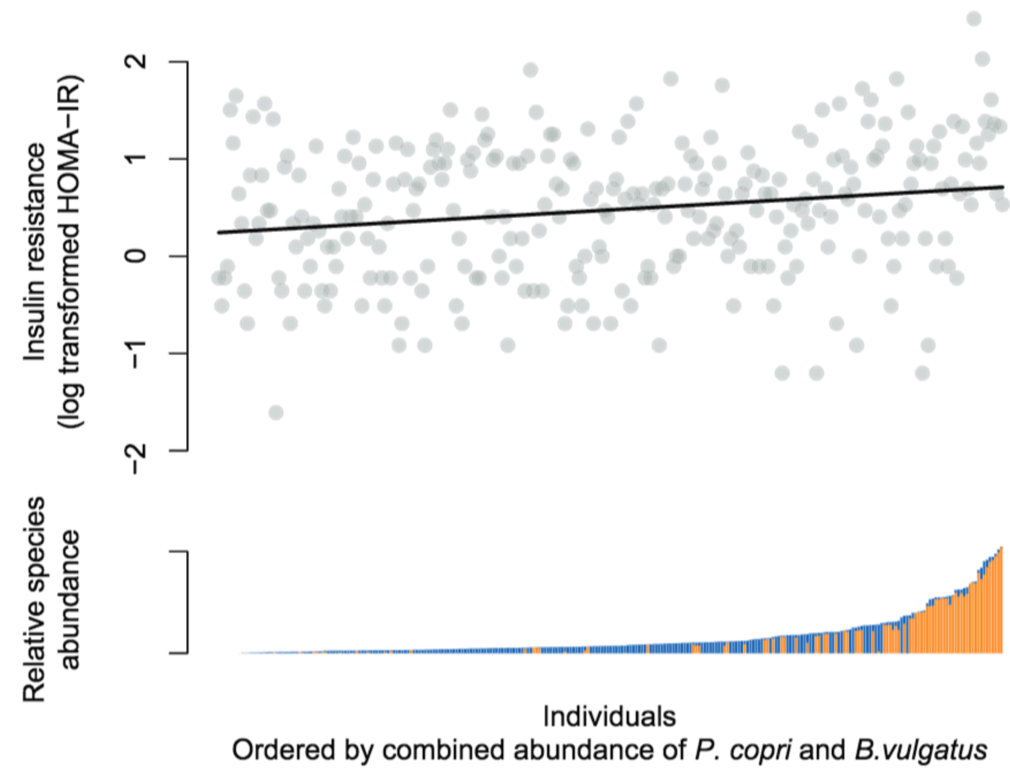


Pedersen, H. K. et al. *Nature*, **2016**, *535*, 376

Case Study: BCAAs and Insulin Sensitivity

To determine responsible species for these functional modules, ***rerun the KEGG analysis omitting genes for a single species each time***





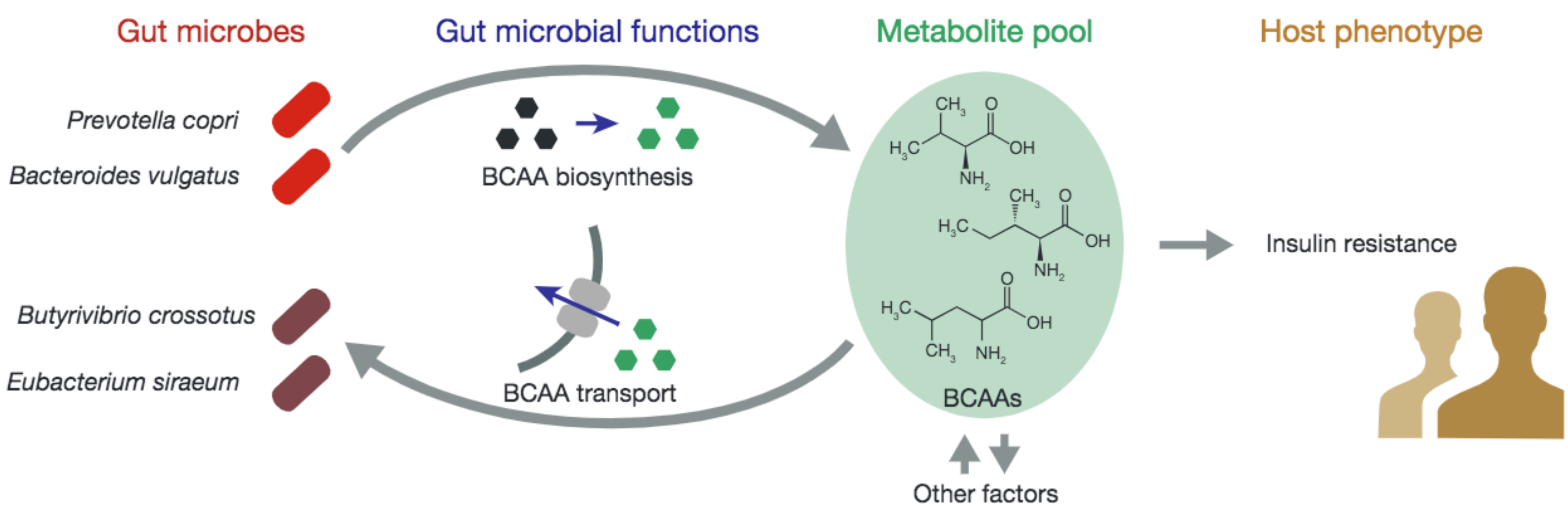
P. copri and B. Vulgatus largely drive the association between insulin resistance and BCAA biosynthetic modules

Pedersen, H. K. et al. *Nature*, **2016**, *535*, 376

Case Study: BCAAs and Insulin Sensitivity

Microbial functional analysis additionally recognized depletion of genes encoding for bacterial BCAA uptake (and their associated bacterial species)

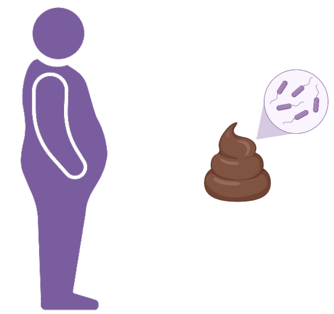
Increased BCAA pool



Pedersen, H. K. et al. *Nature*, **2016**, *535*, 376

Case Study: BCAAs and Insulin Sensitivity

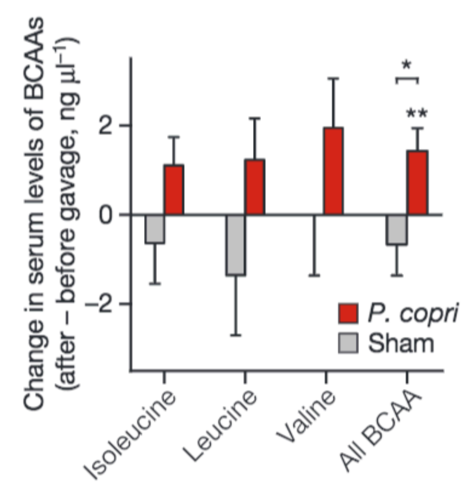
Increased BCAA levels in mice following fecal transplantation from insulin-resistant individuals

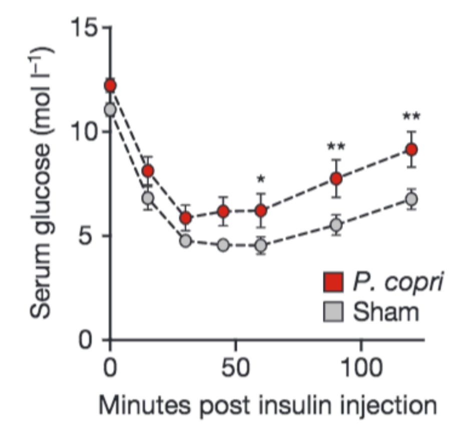


take fecal sample from IR patient

feed fecal sample to mouse

P. copri





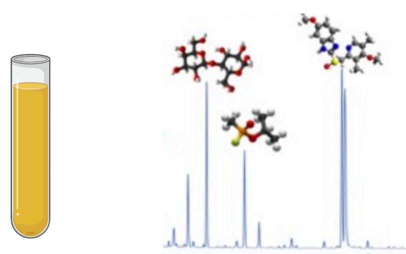
Reduced insulin sensitivity and increased levels of BCAA when mice fed P. copri

Ridaura, V. K. et al. *Science* **2013**, *341*, 1241214

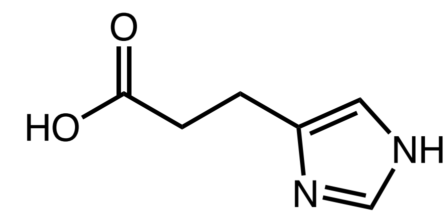
Pedersen, H. K. et al. *Nature*, **2016**, *535*, 376

Case Study: Imidazole Propionate and Insulin Resistance

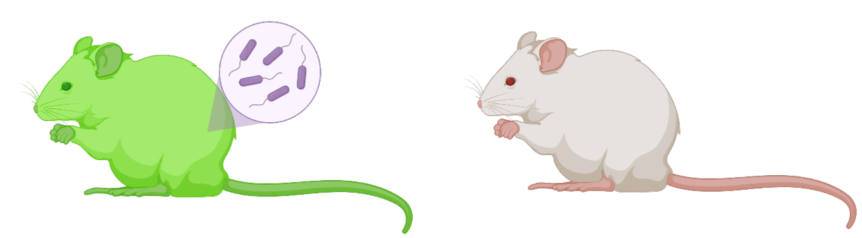
Imidazole propionate has been shown to impair insulin signaling



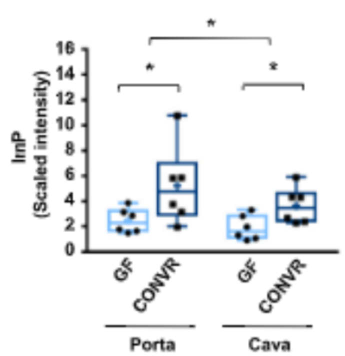
Perform untargeted metabolomics on plasma of patients with and without T2D



Find four AA-derived metabolites in higher concentrations in T2D patients



To focus on microbial metabolites only, repeat metabolomics on germ-free and conventional mice



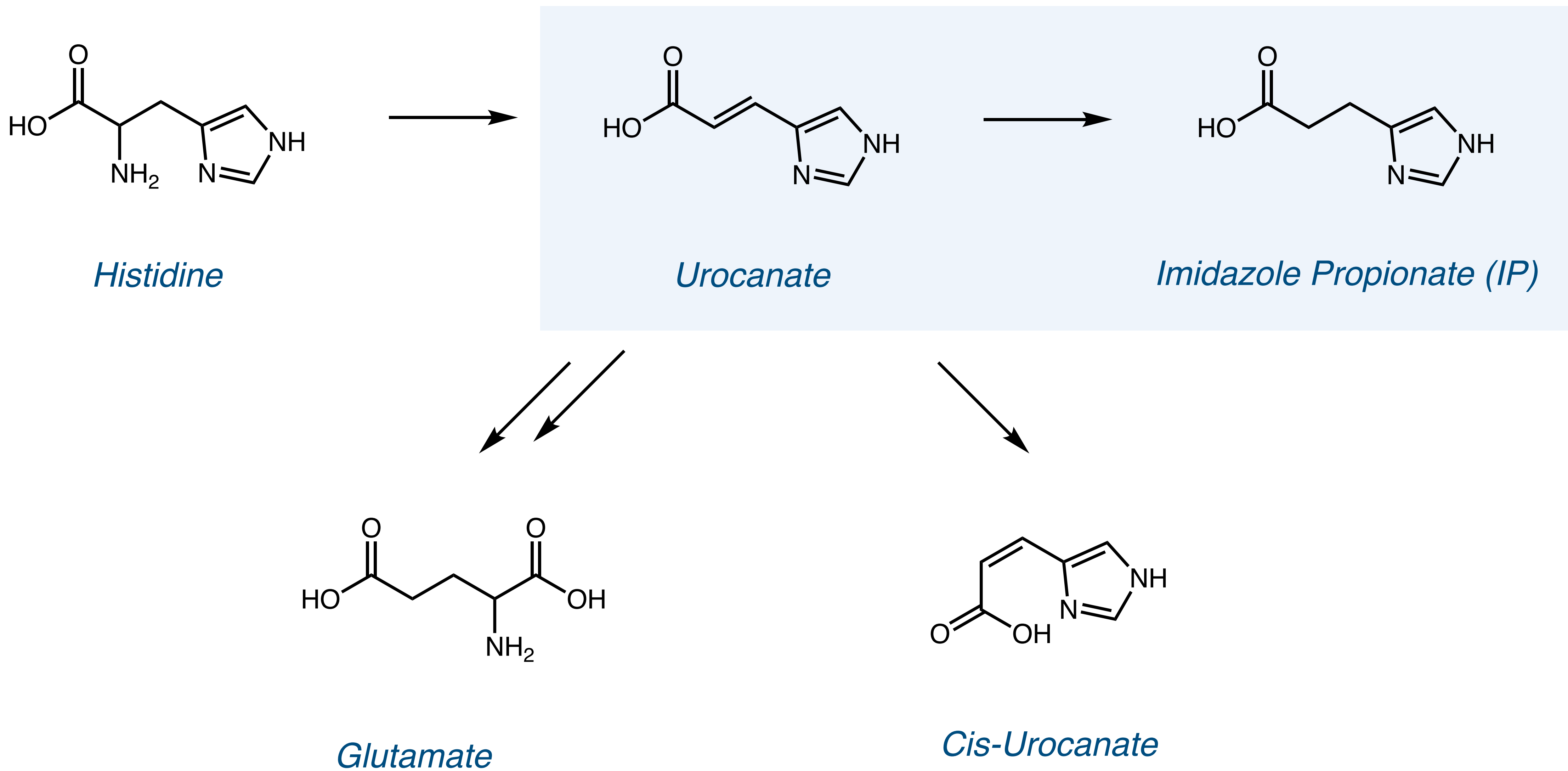
Only imidazole propionate is present in higher concentrations

Koh, A. et al. *Cell* **2018**, *175*, 947

Case Study: Imidazole Propionate and Insulin Resistance

Put histidine in an *in vitro* gut simulator, ***see IP only with T2D microbes***

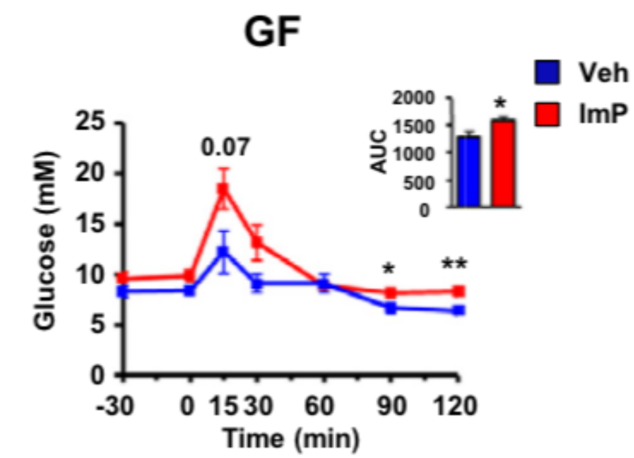
Suggests ***type 2 diabetes-associated microbiota shunts urocanate to a lesser-known pathway to produce imidazole propionate***

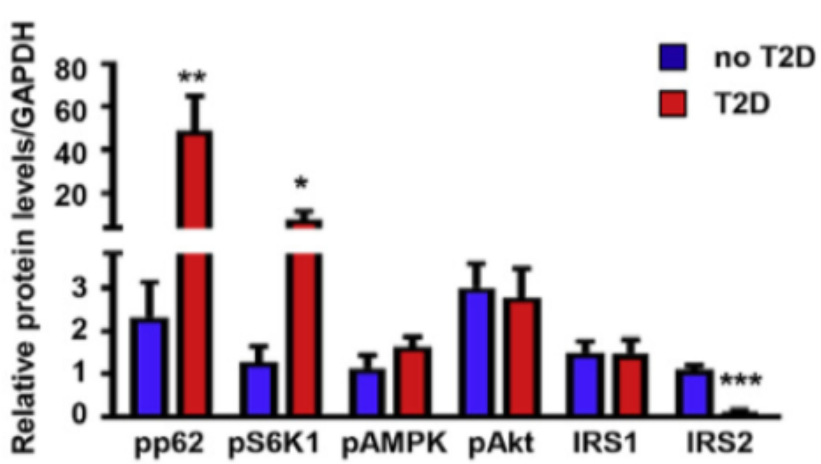


Koh, A. et al. *Cell* **2018**, *175*, 947

Case Study: Imidazole Propionate and Insulin Resistance

Imidazole propionate injected into germ free mice → ***Imidazole propionate worsened glucose intolerance and insulin signaling***





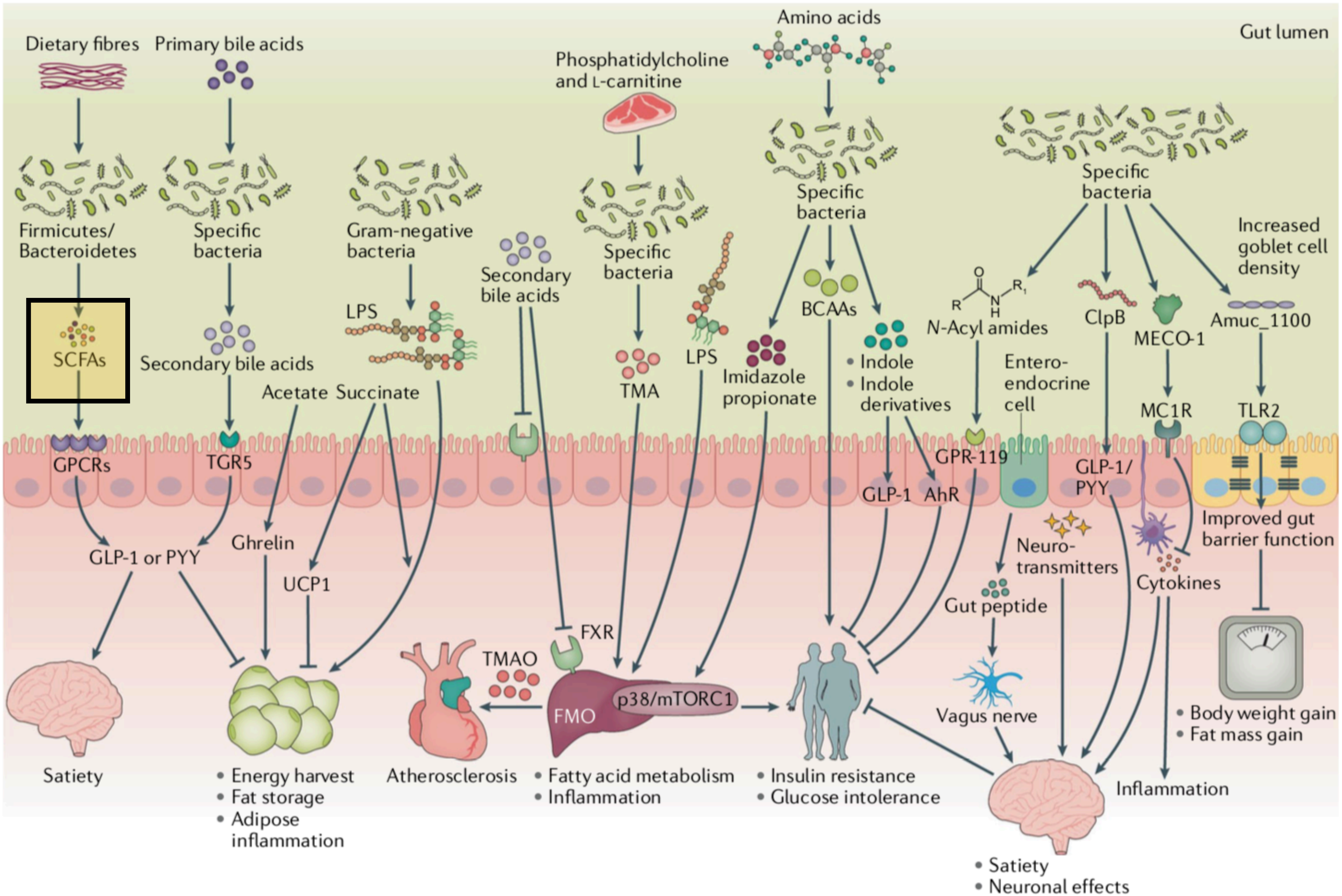
Overexpression of the mTORC1-mediated insulin signaling pathway was found in liver tissue of T2D individuals

↓

Show that IP inhibits insulin signalling through mTOR (rapamycin inhibits this effect)

Koh, A. et al. *Cell* **2018**, *175*, 947

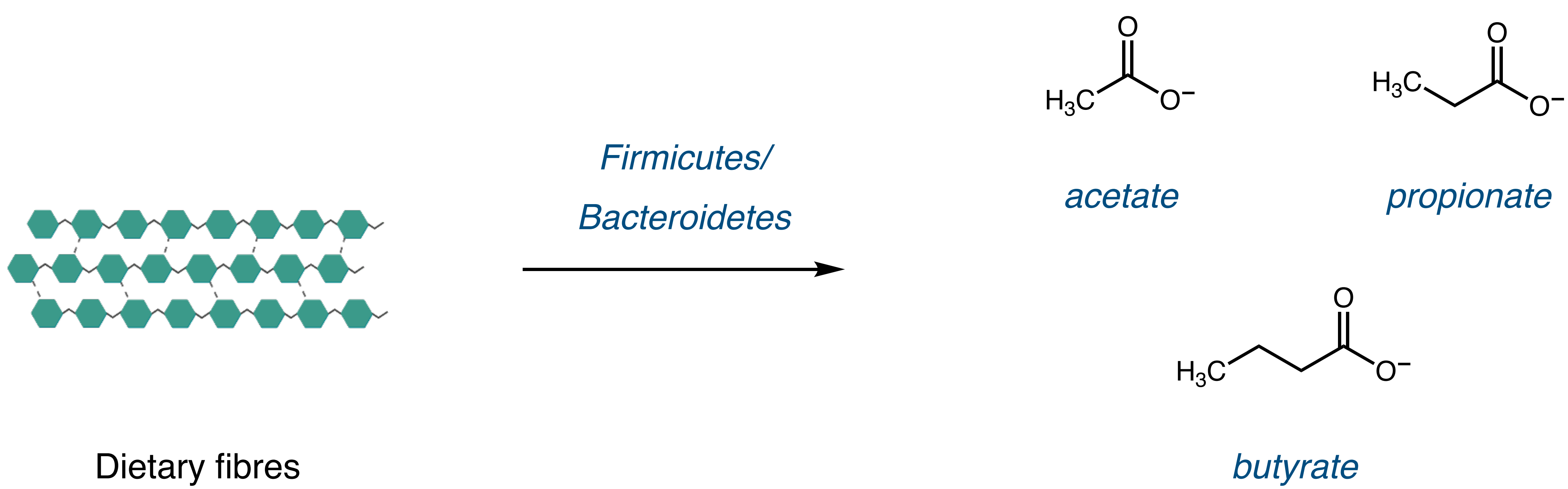
Microbial Metabolites affect Metabolic Health



Pedersen, O.; Fan, Y. *Nat. Rev. Microbiol.* **2021**, *19*, 55

SCFAs and Energy Homeostasis and Body Adiposity

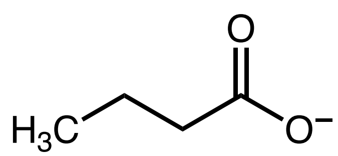
Short Chain Fatty Acids → Monocarboxylic acids with six or fewer carbon atoms produced by the microbiota upon fermentation of indigestible polysaccharides



Short chain fatty acids have effects on satiety, energy harvest, glucose homeostatis, fat storage and inflammation

Pedersen, O.; Fan, Y. *Nat. Rev. Microbiol.* **2021**, *19*, 55

Case Study: SCFAs and Energy Homeostasis and Body Adiposity



Tributyrin

butyrate precursor drug

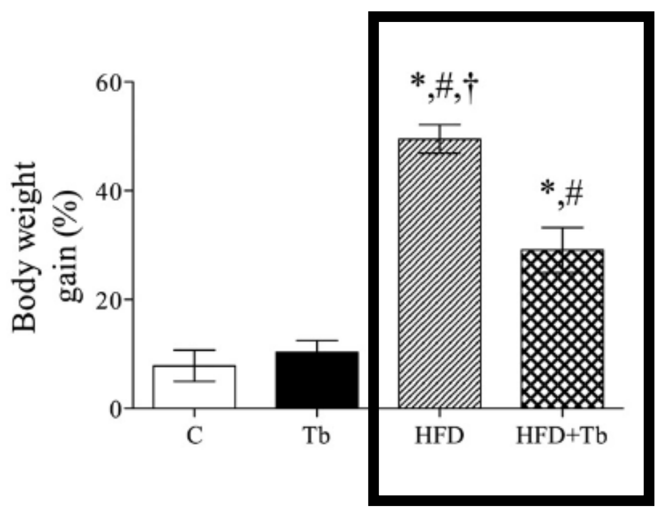
High fat diet (HFD)

(+tributyrin)

Standard diet

(+tributyrin)

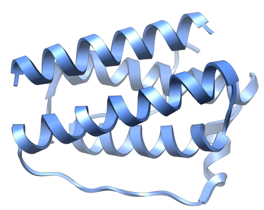
Mice treated with butyrate precursor drug (tributyrin) are ***protected from diet-induced obesity and insulin resistance***



	C	Tb	HFD	HFD+Tb
Triglycerides, mg/dl	43.2 ± 2.6	45.3 ± 3.7	56.7 ± 3.8*#	50.4 ± 4.5
Cholesterol, mg/dl	132.2 ± 6.8	129.8 ± 4.8	183.0 ± 8.3*#	161.2 ± 6.8
LDL cholesterol, mg/dl	67.3 ± 5.5	63.6 ± 6.1	111.3 ± 6.4*#	97.1 ± 9.4
HDL cholesterol, mg/dl	63.3 ± 3.7	58.1 ± 2.1	62.1 ± 3.8	58.2 ± 3.1
NEFA, mM	0.30 ± 0.03	0.32 ± 0.03	0.45 ± 0.05*#†	0.27 ± 0.03
AST, U/ml	111.8 ± 15.9	114.3 ± 9.9	103.8 ± 15.0	102.9 ± 15.1
ALT, U/ml	30.2 ± 10.0	36.5 ± 9.2	34.0 ± 6.5	29.9 ± 4.4
Leptin, pg/ml	4,061 ± 744	6,512 ± 786	20,944 ± 2145*#†	15,642 ± 1,178*#
Resistin, pg/ml	3,788 ± 391	3,950 ± 353	7871 ± 595*#†	6,024 ± 447*#
Fasting glucose, mM	9.48 ± 0.40	8.86 ± 0.34	10.86 ± 0.35*#†	9.48 ± 0.35
Insulin, ng/ml	0.37 ± 0.04	0.47 ± 0.07	1.01 ± 0.08*#†	0.75 ± 0.09*#
HOMA-IR	21.92 ± 3.38	26.84 ± 3.85	75.70 ± 5.80*#†	45.77 ± 6.31

Vinolo, M. A. R. et al. *Am. J. Physiol. Endocrinol. Metab.* **2012**, *303*, E272

Case Study: SCFAs and Leptin



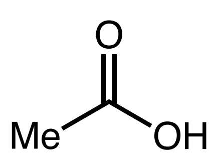
Leptin

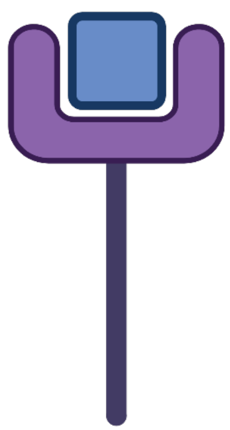
Brain

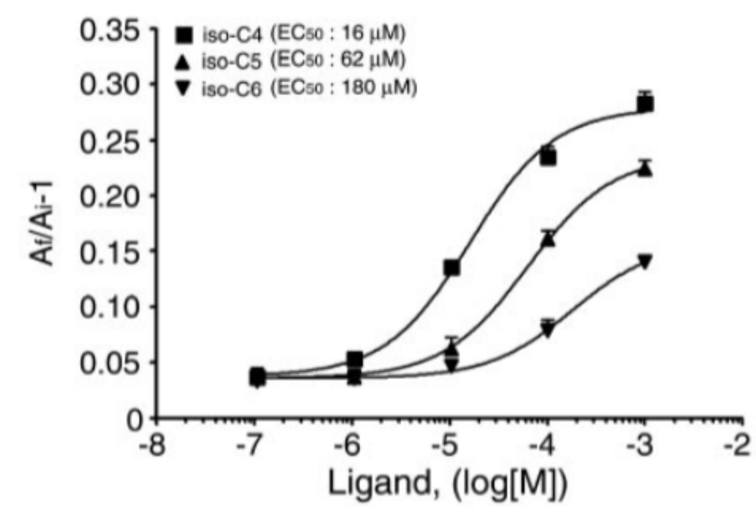
Stop eating

Response

SCFAs are linked to leptin, an anorexigenic hormone that suppresses food intake







Unexpectedly find that acetic acid (solvent) during ligand screening for human GPR41 receptor

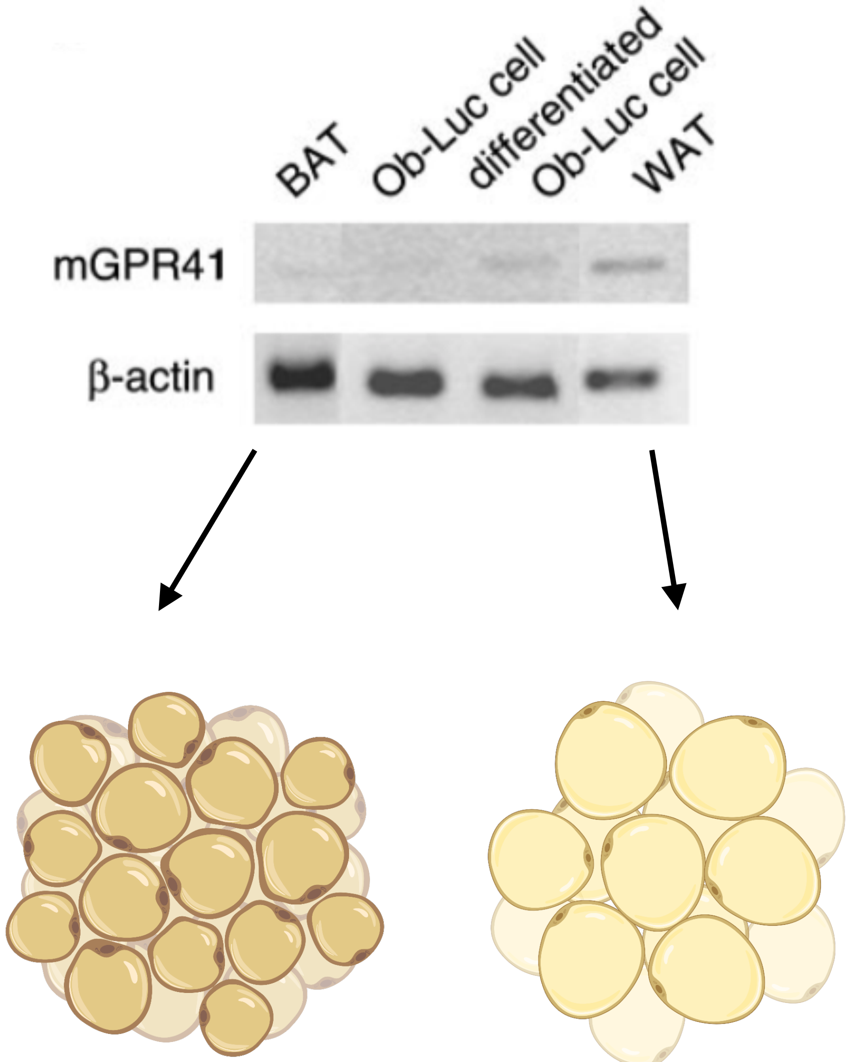
Find that C2-C6 SCFAS can bind to GPR41

Xiong, Y.; Miyamoto, N.; Shibata, K.; Valasek, M. A.; Motoike, T.; Kedzierski, R. M.; Yanagisawa, M. *PNAS* **2004**, *101*, 1045

Case Study: SCFAs and Leptin

Found that GPR41 mRNA is expressed in white (WAT) but not brown adipose tissue (BAT)

As leptin production is much higher in white adipose tissue, looked at effect of SCFAs on leptin

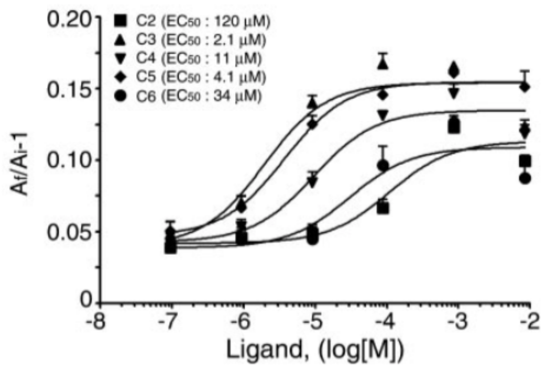


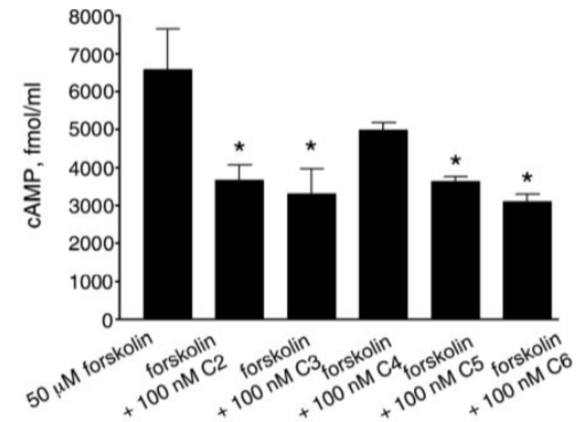
Brown adipose tissue

White adipose tissue ***(higher leptin production)***

Xiong, Y.; Miyamoto, N.; Shibata, K.; Valasek, M. A.; Motoike, T.; Kedzierski, R. M.; Yanagisawa, M. *PNAS* **2004**, *101*, 1045

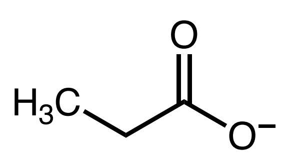
Case Study: SCFAs and Leptin



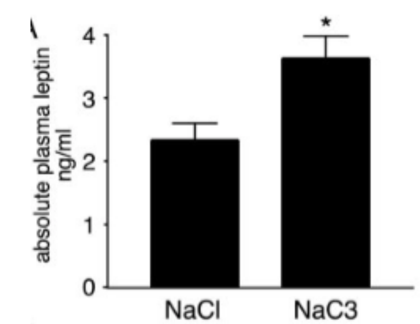


Using a luciferase assay, find that SCFAs stimulate leptin production in Ob-Luc cells

Increased luciferase activity with propionic acid in cells overexpressing GPR41



Propionate



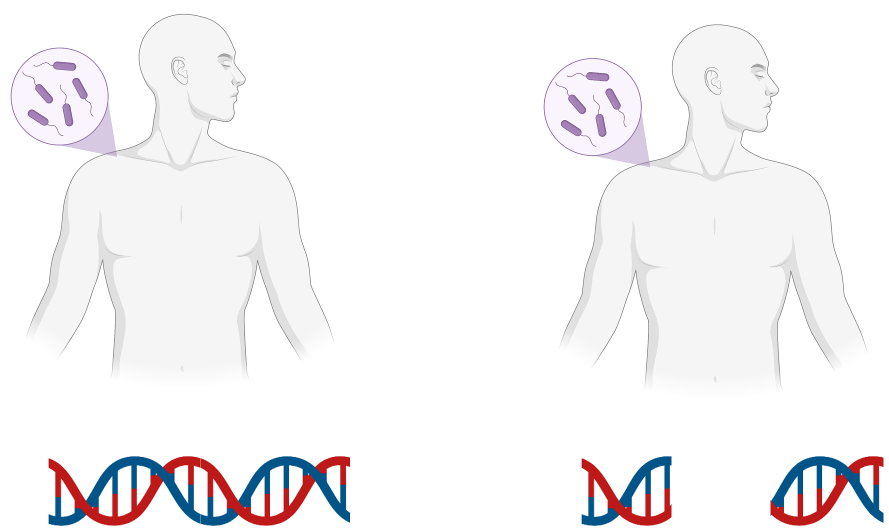
Oral administration of propionate increases circulating leptin levels in vivo

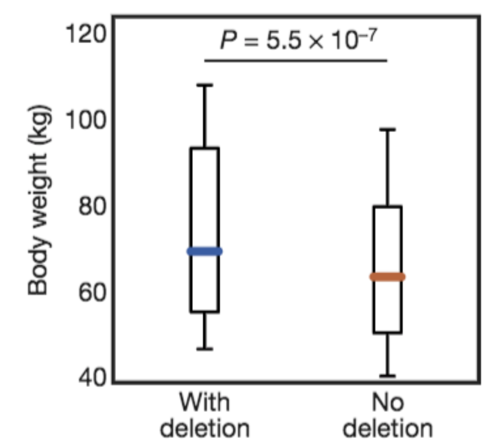
Xiong, Y.; Miyamoto, N.; Shibata, K.; Valasek, M. A.; Motoike, T.; Kedzierski, R. M.; Yanagisawa, M. *PNAS* **2004**, *101*, 1045

Case Study: SCFAs and Energy Homeostasis and Body Adiposity

Look for DNA segments that are deleted from bacteria in some individuals or present in a variable number of copies in others

Look for associations between these structural variations





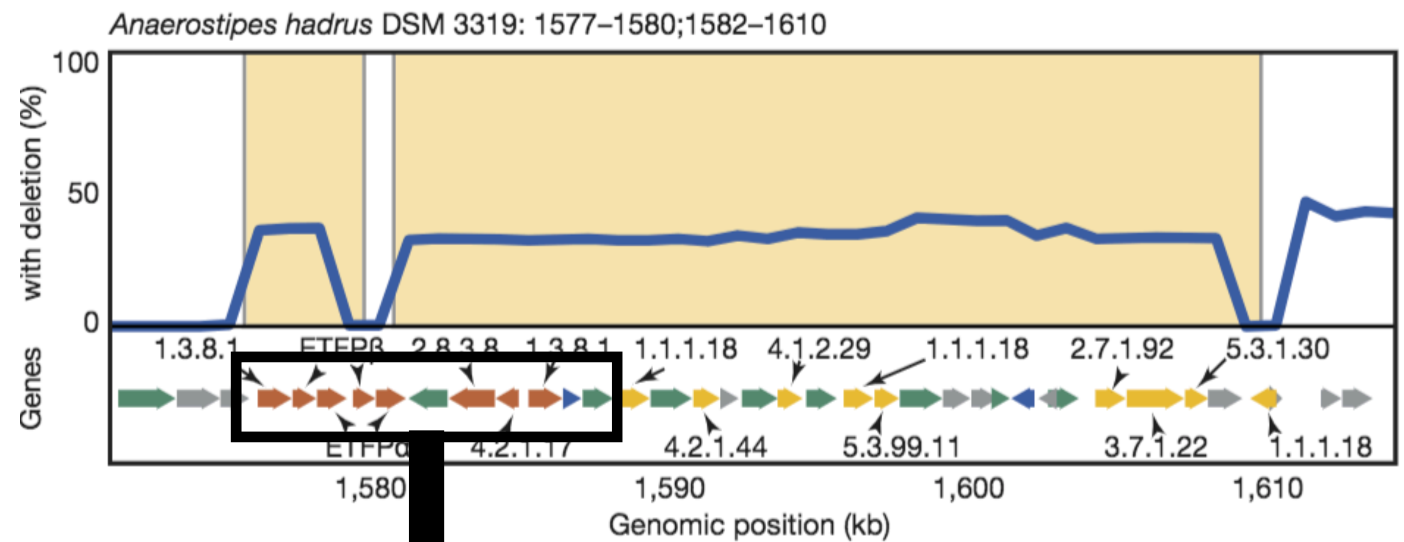
Structural variations in *Anaerostipes Hadrus* genome shows inverse relationship with body weight, waist circumference and BMI

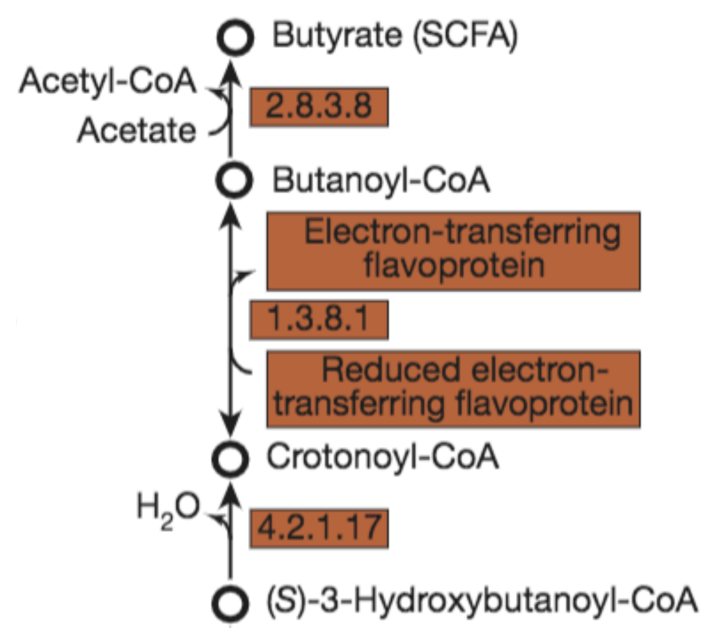
Zeevi, D. et al. *Nature* **2019**, *568*, 43

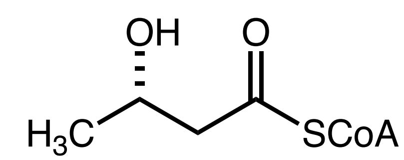
Case Study: SCFAs and Energy Homeostasis and Body Adiposity

These structural variations encode metabolic modules for metabolizing 3-hydrobutanoyl-CoA to butyrate

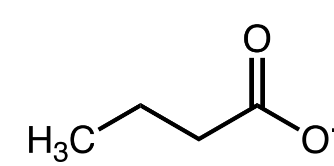
Potential mechanistic link between these metabolic pathways and host health







3-hydrobutanoyl-CoA



Butyrate

Zeevi, D. et al. *Nature* **2019**, *568*, 43

Outline

Overview of Gut Microbiome

Microbial Metabolites and Metabolic Health

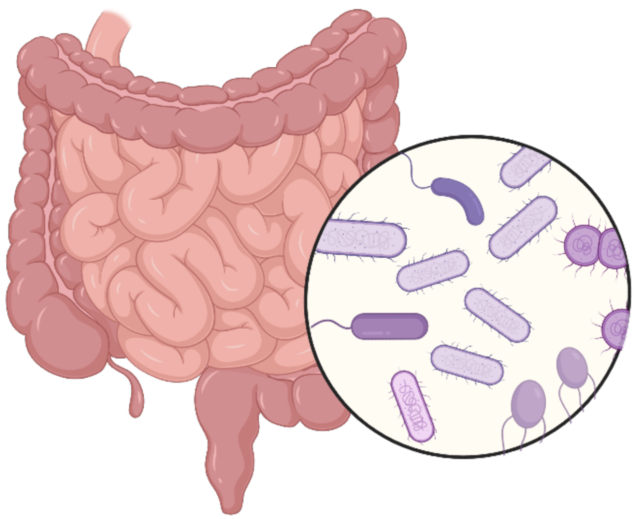
- BCAAs
- Imidazole Propionate
- SCFAs

Gut Microbiome and Metabolic Disease

- Obesity
- Cardiovascular Disease

Interventions

- Diet
- Drugs and Pre/Pro/Postbiotics
- Bioengineered Commensals
- Fecal Microbiota Transplantation



Weight of Germ-Free Mice vs. Conventional Mice

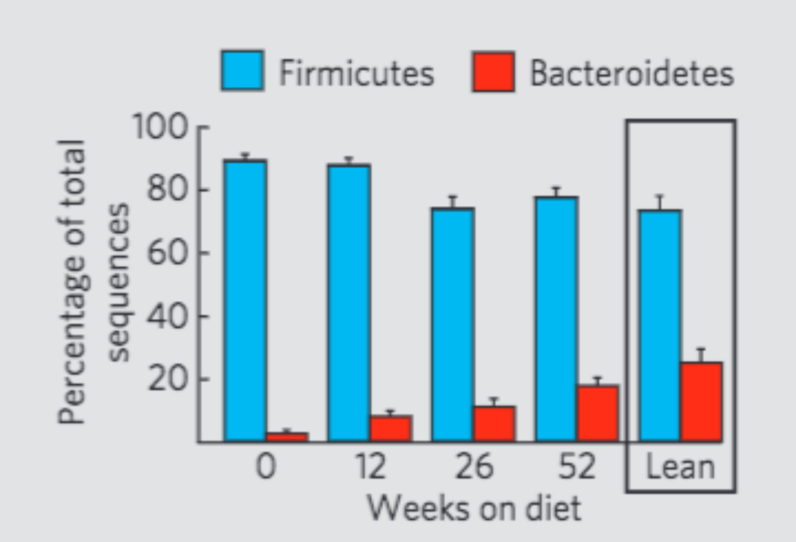
Germ-free mice (no microbes) can eat 30% more calories than conventional mice ***and not gain weight***

Microbiota are important for obesity and weight gain

Coates, M. E. *Laboratory Animals* **1975**, *9*, 275

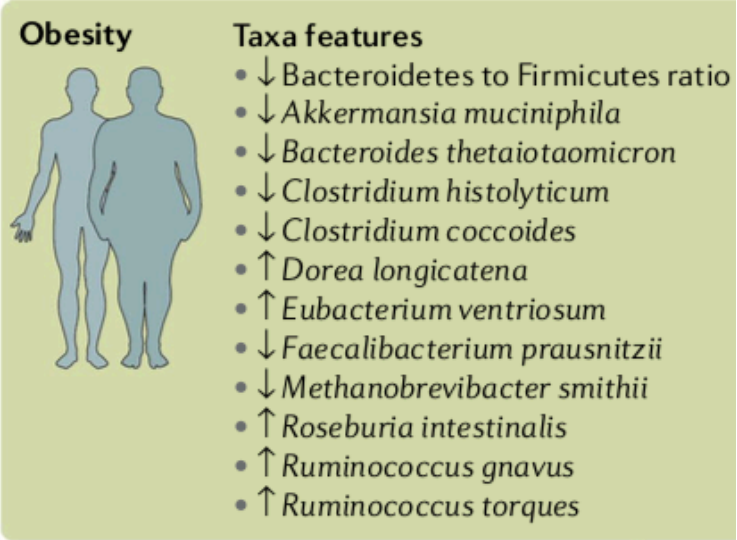
Composition of the Microbiome differs between Obese and Lean Individuals

The ***composition of the microbiome differs between obese and lean individuals*** in humans



Firmicutes:Bacteroidetes ratio decreases with weight loss

At the species level, levels of multiple gut microbiota change between obese and lean individuals



Ley, R. E.; Turnbaugh, P. J.; Klein, S.; Gordon, J. I. *Nature* **2006**, *444*, 1022
Pedersen, O.; Fan, Y. *Nat. Rev. Microbiol.* **2021**, *19*, 55

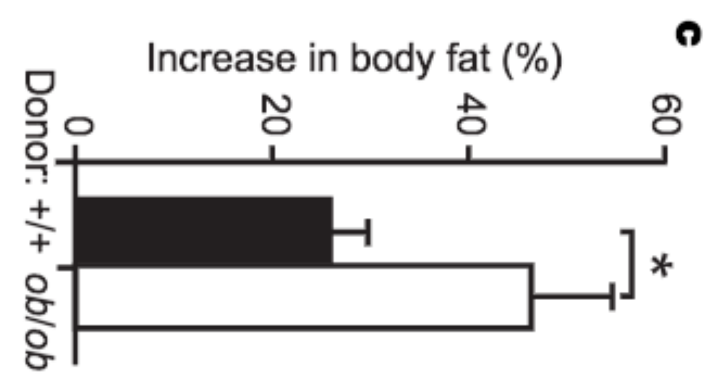
Transfer of Obese Mouse Microbiota

Germ-free (GF) mouse
(no microbes)

Colonization of GF mouse with lean or obese mouse microbes

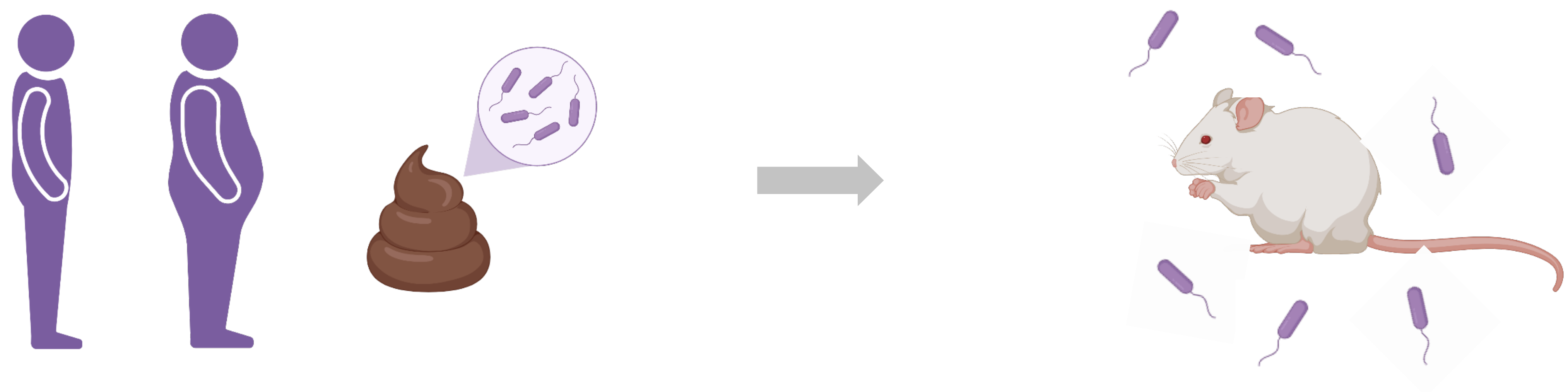
Compare GF mice colonized with lean or obese microbes

Colonization with obese mouse microbes over lean mouse leads to ***significant increase in body fat%***



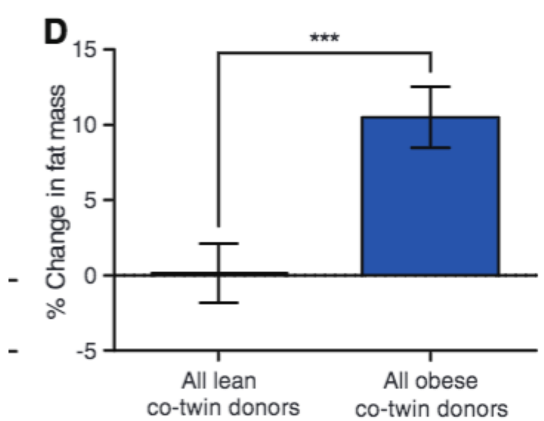
Turnbaugh, P. J.; Ley, R. E.; Mahowald, M. A.; Magrini, V.; Mardis, E. R.; Gordon, J. I. *Nature* **2006**, *444*, 1027

Effect of Fecal Transplant

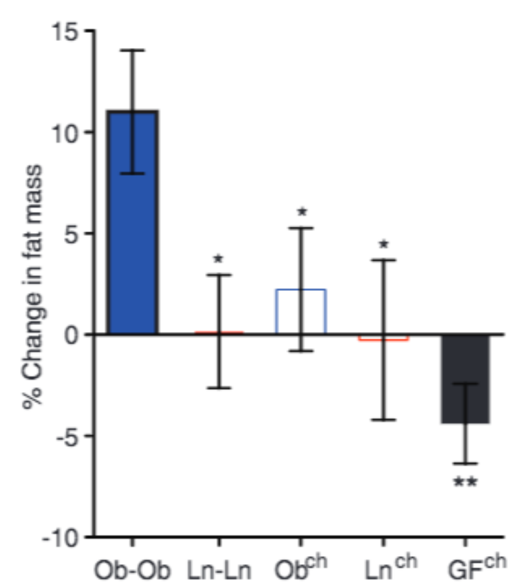


Took fecal samples from lean and obese twin

Colonized GF mice with these microbiota by fecal transplantation



Increased total body and fat mass were transmissible



Cohousing lean and obese mice prevents increased adiposity

(mice are coprophagic - feces eating)

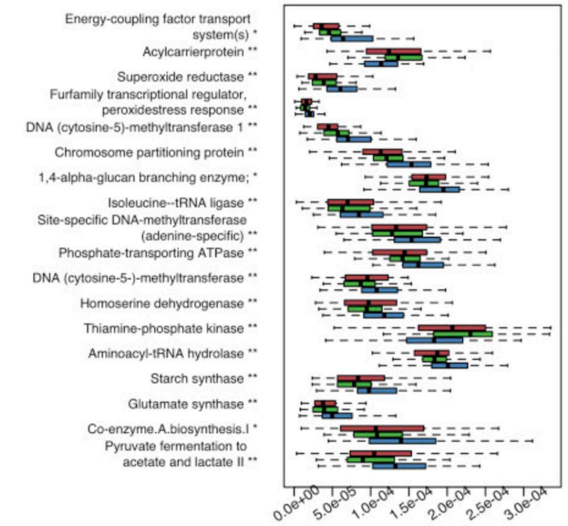
Ridaura, V. K. et al. *Science* **2013**, *341*, 1241214

Obesity is associated with a Decreased Capacity for Unidirectional Conjugation

Microbiota from stool samples of obese and lean individuals

Extract and sequence DNA

Compare DNA with database



Identify genes, pathways and relative frequencies in sample

Obesity associated with ***decreased capacity for transfer of genetic material*** between bacteria and ***reduction in superoxide reductase***

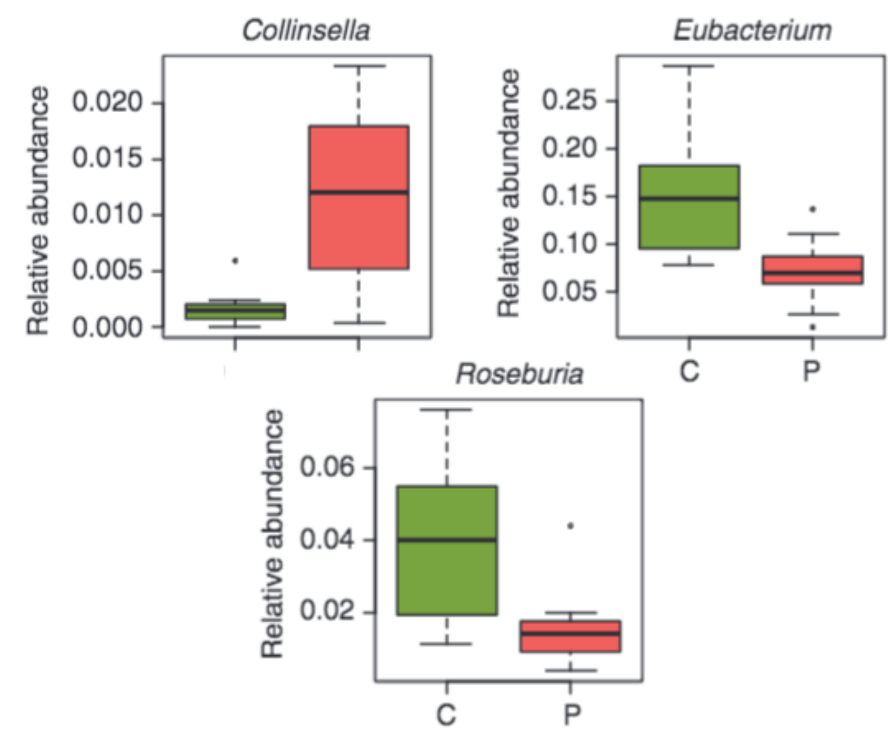
Intestinal oxidative stress

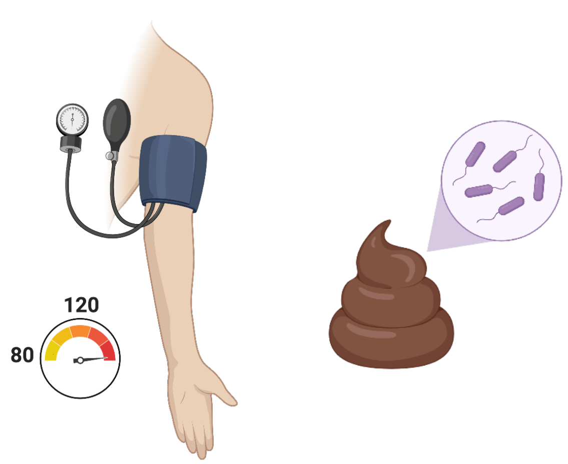
Thingholm, L. B. et al. *Cell Host Microbe* **2019**, *26*, 252

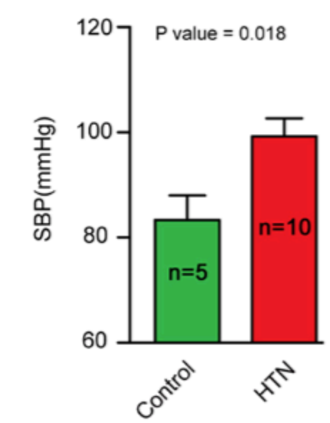
Cardiovascular Disease

Changes in gut microbiota structure and function in those with symptomatic atherosclerosis

Enrichment in genus *Collinsella* and depletion of *Roseburia* and *Eubacterium*



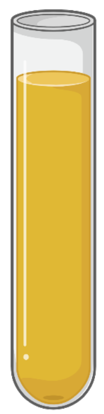


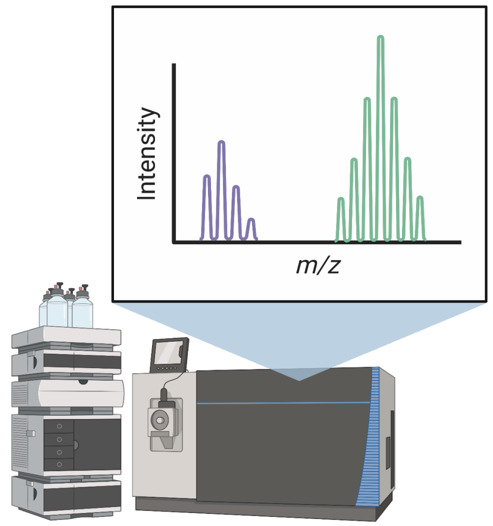


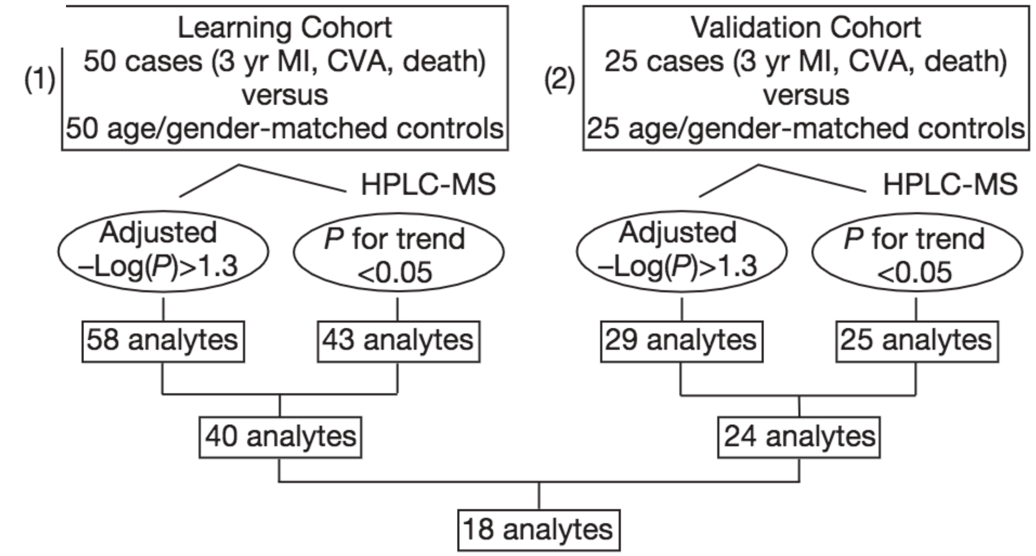
Fecal microbiota transplantation from hypertensive patients to GF mice leads to elevated blood pressure

Jie, Z. et al. *Nat. Commun.* **2012**, *3*, 1245

TMAO and Cardiovascular Disease





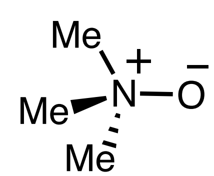


Blood plasma from controls and CVD patients

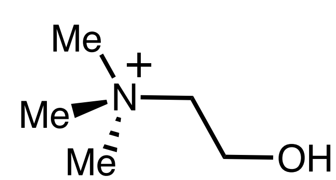
LC/MS analysis to define analyses associated with cardiac risk

Applying acceptability criteria gave 18 analytes

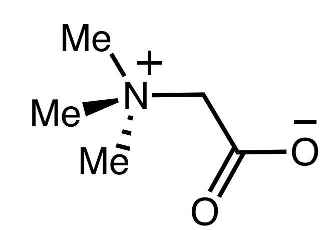
3 of the 18 analytes showed significant correlations between one another



TMAO



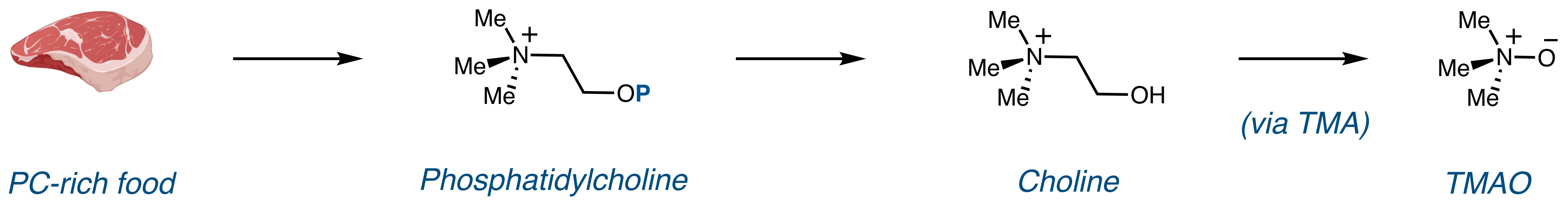
Choline



Betaine

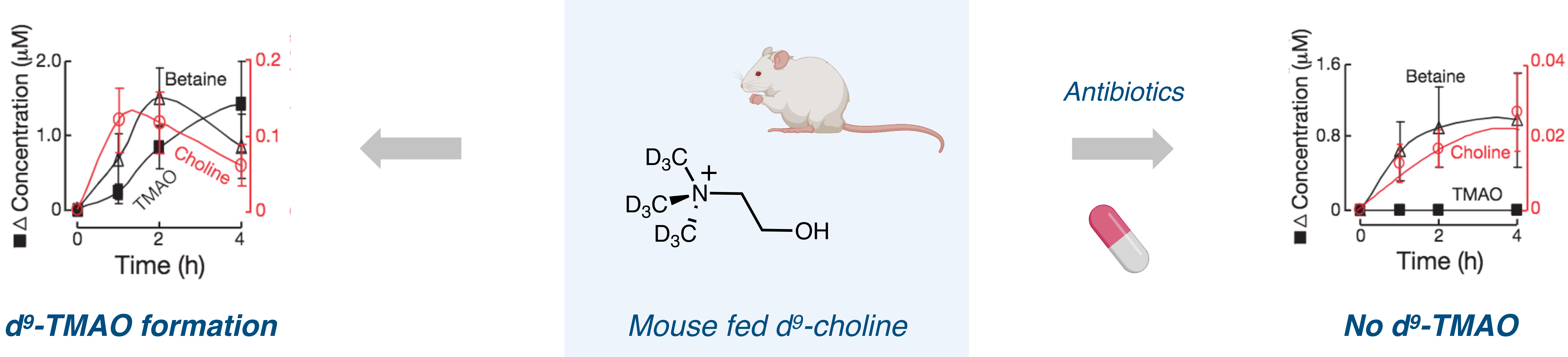
Wang, Z. et al. *Nature* **2011**, *472*, 57

TMAO and Cardiovascular Disease



Foods rich in the lipid phosphatidylcholine (e.g eggs, red meat, fish) thought to be major dietary sources of choline

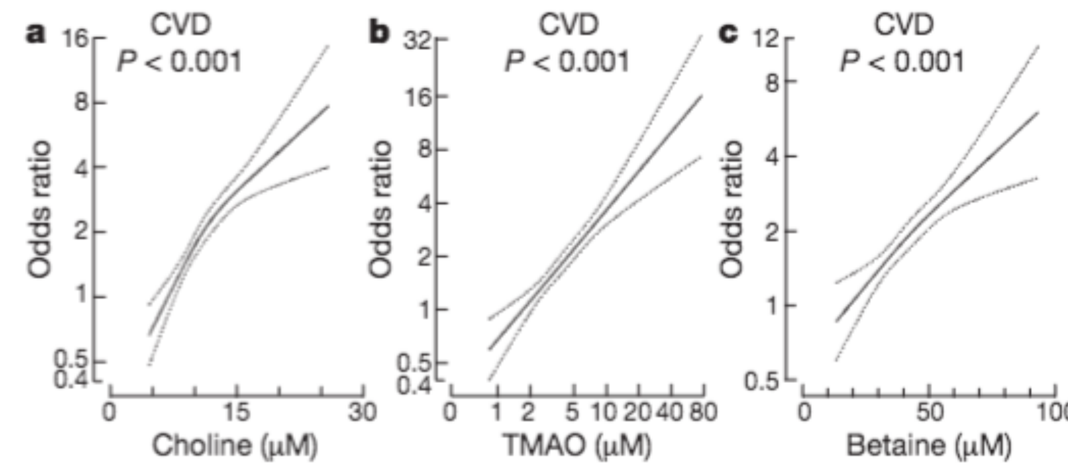
Proposed that ***intestinal microflora have a role in TMAO formation from dietary free choline***

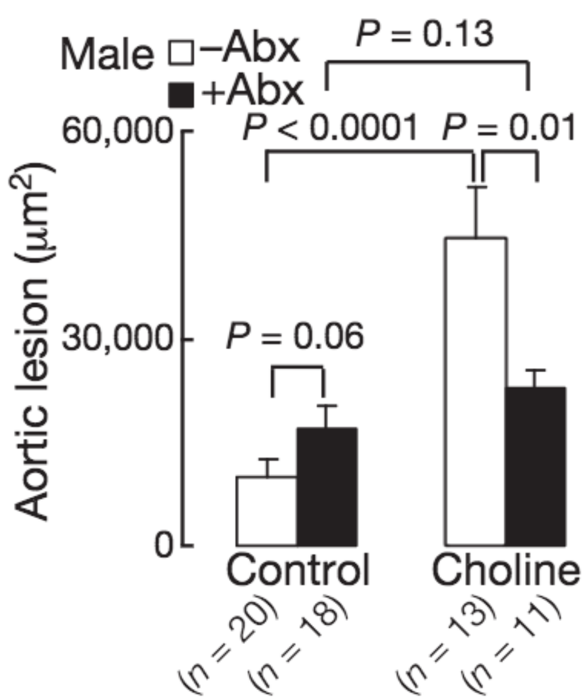


Wang, Z. et al. *Nature* **2011**, *472*, 57

TMAO and Cardiovascular Disease

Plasma levels of choline, TMAO and betaine were ***associated with atherosclerosis risk in humans***





Atherosclerosis-prone mouse fed TMAO or choline

Promoted atherosclerosis (x3!)

Mouse pretreated with antibiotics

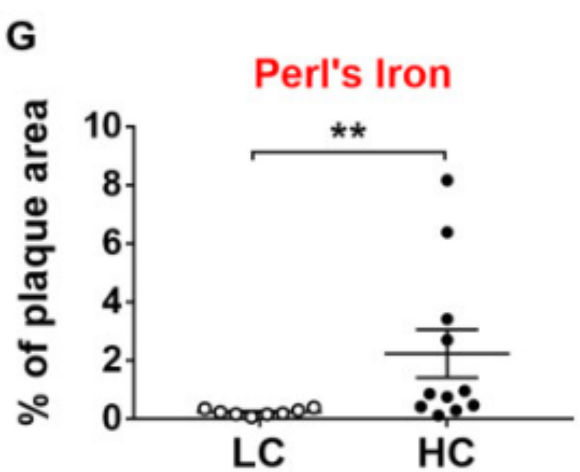
No effect on atherosclerosis

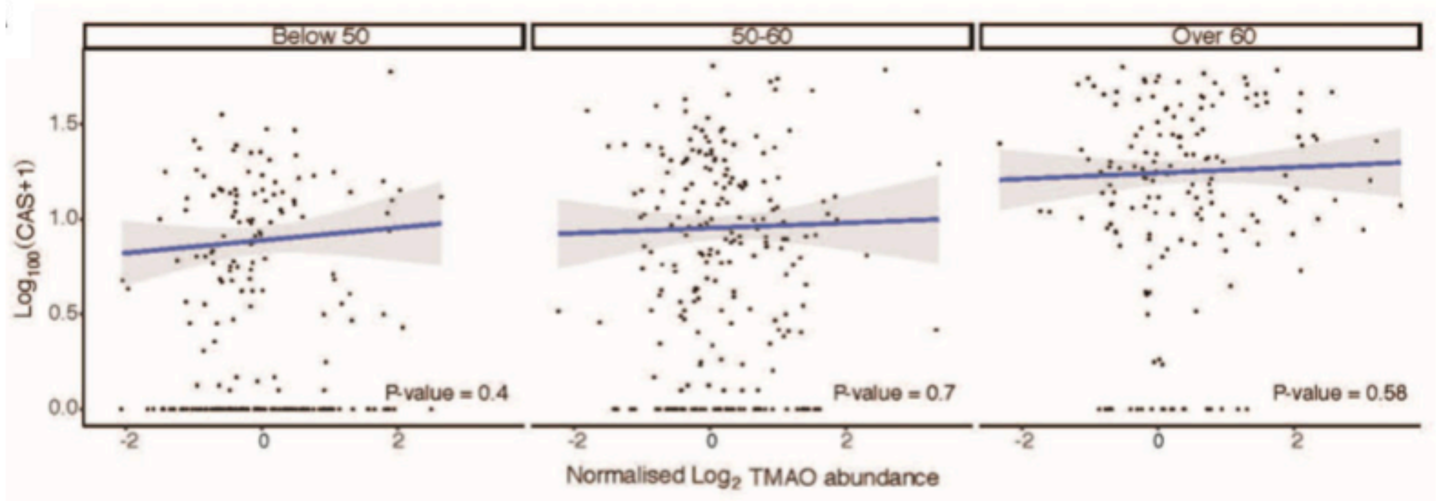
Wang, Z. et al. *Nature* **2011**, *472*, 57

TMAO and Cardiovascular Disease

Role of TMAO in promoting atherosclerosis is controversial

However, TMAO has been shown to be an excellent biomarker for predisease





Choline-rich diet led to ***plaque instability*** in prone mice ***but not atherosclerosis***

TMAO aggravates atherogenesis in prone individuals primarily though plaque instability

Koay, Y. C. *Cardiovasc. Res.* **2020**, 8

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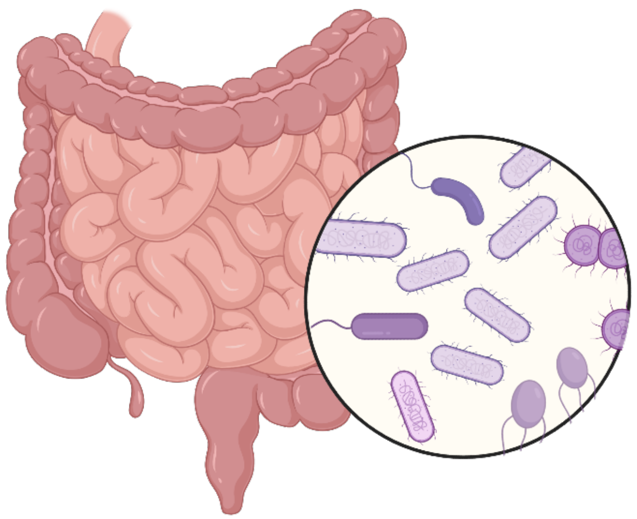


Interventions

Untargeted | **Targeted**

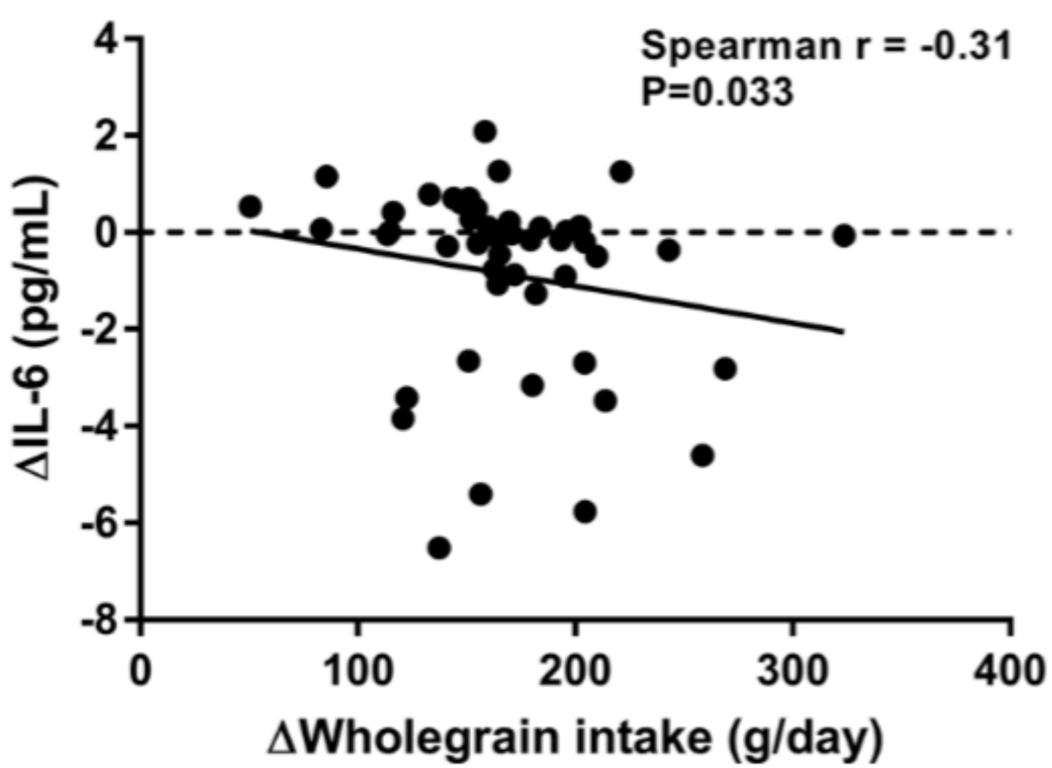
General improvement in microbial composition and function

Specific modification in metabolism-related gut microbiota

Pedersen, O.; Fan, Y. *Nat. Rev. Microbiol.* **2021**, *19*, 55

Diet

Diets high in saturated or monounsaturated fat negatively influence the microbiota whereas diets high in polyunsaturated fat are neutral



High polysaccharide diets are beneficial and lead to:

- altered gut microbiota with increased faecal, serum of urine concentrations of SCFAs
- weight loss
- improvements of cytokine and metabolome profiles

Roager. H. M. et al. *Gut* **2019**, *68*, 83
Wolters, M. et al. *Clin. Nutr.* **2018**, *38*, 2504

Diet

Diet high in vegetable fibers

(low in animal fat/protein)

Indigestable polysaccharides

Fermented by beneficial bacteria

Short chain fatty acids (SCFAs)

Beneficial effects to host

Diet high in animal fat/protein

(low in vegetable fibers)

No fermentable polysaccharides, microbes switch to ***amino acids***

Fermented by harmful bacteria

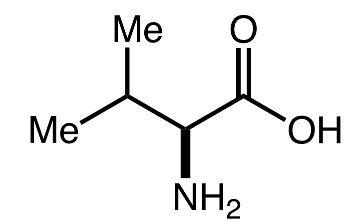
Acidic products => increase in pH

Causes leakage of molecules into blood triggering inflammation and IR

Pedersen, O.; Fan, Y. *Nat. Rev. Microbiol.* **2021**, *19*, 55

Pre-, Pro-, Postbiotics

Pre-, pro- and postbiotics deliver microorganisms or substrates that provide health benefit to host



Prebiotics

Substrate

Probiotics

Micro-organisms

Postbiotics

Metabolites

(+ microbes sometimes)

Probiotic strains Lactobacillus, Bifidobacterium and Saccharomyce have a long history of safe and effective use

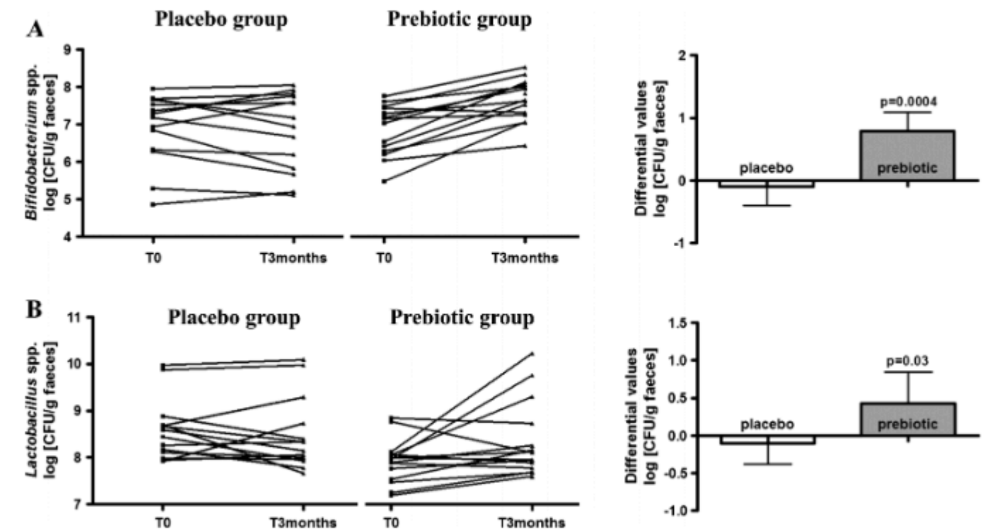
Clinical indications for multiple diseases (e.g IBS, H.pylori) although more studies needed (no official recommendation)

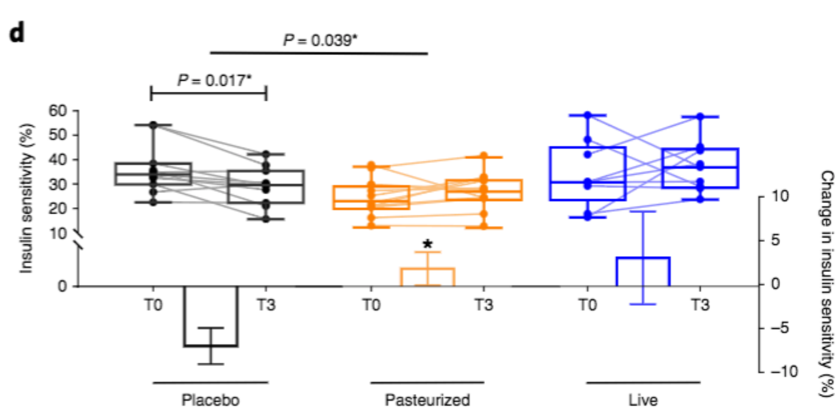
Pedersen, O.; Fan, Y. *Nat. Rev. Microbiol.* **2021**, *19*, 55

Pre-, Pro-, Postbiotics

Prebiotic and post studies lag behind probiotic studies, although many promising studies

Oligo-fructose-enriched inulin prebiotics alter the intestinal microbiota and modestly reduce body weight, adiposity and inflammatory markers in obese children





	Placebo	Pasteurized	Live
Mean difference *P* value (Mann–Whitney *U*-test)	−6.79 ± 2.07	2.13 ± 1.89 0.002*	3.24 ± 5.28 0.095
Mean difference from placebo Mean difference from placebo (%)	– –	8.92 ± 1.89 28.62 ± 7.02%	10.03 ± 5.28 42.42 ± 20.44%

Pasteurized A.muciniphila and its membrane protein Amuic_1100 demonstrated positive effects on markers of human metabolism

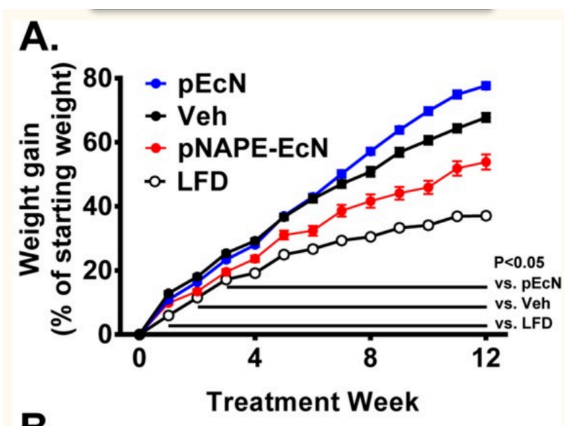
Depommier, C. et al. *Nat. Med.* **2019**, *25,* 1096

Dewulf, E. M. *Gut* **2013**, *62*, 1112

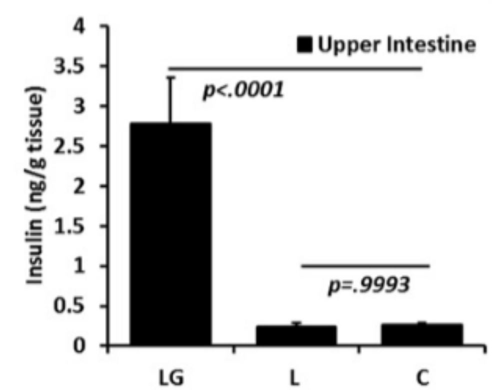
Bio-engineered Commensals

Bio-engineered commensals → Genetically modified microbes

Recent promising examples:



E. Coli overexpressing N-acylphosphatidylethanolamine alleviates diet-induced obesity and insulin resistance



L. Gasseri strain engineered to express and secrete GIP-1 increased insulin release and reduced hyperglycemia in diabetic mice

Is delivering genetically modified organisms carrying microbial genes to the human gut acceptable?

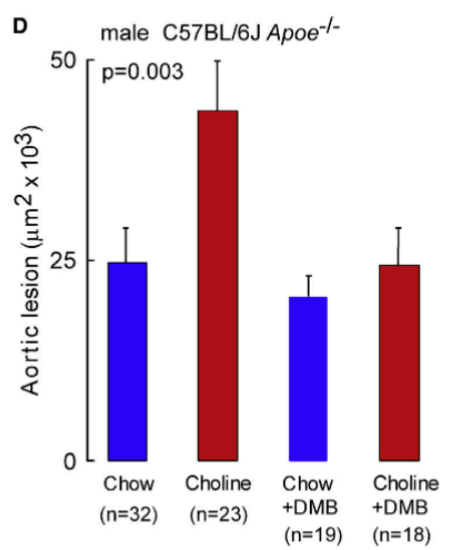
Chen, Z. et al. *J. Clin. Invest.* **2014**, *124,* 3391

Duan, F. F.; Liu, J. H.; March, J. C. *Diabetes* **2015**, *64*, 1794

Drugs Targeting Selected Microbial Metabolism

Targeting specific microbial-synthesized metabolites by delivering tailored drugs - an emerging frontier

TMA inhibition - an early success:



DMB, an inhibitor of TMA, inhibited choline diet-enhanced atherosclerosis in mice

Ongoing clinical trials:

Metabolite	Major physiological effect	Reference for basic science study	Clinical trial (condition, intervention, stage)	Reference for clinical trial
TMAO	Alterations of cholesterol, sterol metabolism and bile acid pool size and composition	[11,14]	Chronic kidney disease, Rifaximin antibiotic, phase 4	NCT03718988
	Platelet hyperreactivity and thrombosis risk	[13,18]	Heart failure, Rifaximin vs S. boulardii, phase 2	NCT02637167
			Chronic kidney disease, Sevelamer Carbonate, phase 3	NCT03596749
			Chronic Kidney Disease, AXOS vs Maltodextrine, phase 2	NCT02141815
SCFAs	Hematopoietic alterations through HDACs, gut-brain axis	[24,30,41,42]	Stress and attention, SCFA oral supplementation, N/A	NCT03688854
Butyrate	Epigenetic modulation of inflammation		Schizophrenia	NCT03010865
	Increased fatty acid oxidation	[124]	Childhood Obesity: Liver damage and insulin resistance, dietary supplement, Phase 2 and 3	NCT02721953
	Inflammation and oxidative stress	[124,125]	Minor effects on oxidative stress, enemas sodium butyrate, N/A	NCT00696098
Conjugated linoleic acid (CLA)	Fortification of epithelial barrier integrity	[48,49]	obesity, oral supplementation, N/A	PMID: 11592727
N-3 long chain fatty acid	Attenuation of inflammation	[126]	Ulcerative Colitis, oral supplement, Phase 3	PMID: 15822041
Indole-3-carbinol	Ahr mediated control of intestinal epithelial cells proliferation and differentiation	[58]	Squamous Cell Head and Neck Cancer, SCB01A, phase 2	NCT03020823
			Prostate cancer, phase 2/3	NCT00579332
			Breast Cancer, DIM supplementation, phase 3	NCT02525159
Indole	Ahr mediated mucosal immunity	[59,61]	Healthy, *L. reuteri* recolonization, N/A	NCT03501082
Indole-3-carbinol	Ahr mediated immunoregulatory effects	[127]	Systemic Lupus Erythematosus, DIM supplementation, phase 1	NCT02483624
			Obesity, Indole 3 carbinol supplementation, phase 2	NCT00988845
Indole-3-propionic acid			Friedreich's Ataxia, VP 20629, phase1	NCT01898884
Indoxyl sulfate	Uremic and vascular toxin	[67]	Chronic kidney disease, AST-120 (Kremezin®), phase 4	NCT01157260
p-cresol	Uremic toxin altering of endothelial cells function and promoting vascular calcification	[70,71]	Chronic Kidney Disease, BENEO synergy1 (inulin/oligofructose), Phase1/2	NCT00695513
			Chronic Renal Failure, Synbiotic Probinul-Neutro®, phase 4	NCT02008331
Taurine	Modulation of gut bile acid metabolism, anti-inflammatory, oxidative stress	[79,80]	Diabetes, oral supplement, N/A	NCT03410537
				NCT01226537
Imidazole propionate	Impairment of the insulin signaling	[84]	type 2 diabetes, high vs low protein diets, N/A	NCT03732690
Retinoic Acid	T-Cell development	[93]	Primary immune thrombocytopenia, ATRA supplement, phase 2	NCT01667263
UDCA	Ameliorate insulin insensitivity		Type 2 diabetes, Ursodiol, phase 2	NCT02033876
	Inhibition of *Clostridium difficile* spore germination and vegetative growth	[105]	Diarrhea, Ursodiol, phase 4	NCT02748616
DCA	Colonic crypt regeneration	[106]	Esophageal Carcinoma, Ursodiol, phase 2	NCT01097304
Flavonoid	Antioxidant and anti-inflammatory properties	[116,117]	Autism Spectrum Disorders, Luteolin, Quercetin and Rutin dietary supplement, phase 2	NCT01847521
			Metabolic Syndrome, chlorogenic acid and luteolin, N/A	NCT03444558
			Alzheimer's diseases or T2DM, polyphenolic extract, phase 1	NCT02502253
N-acyl amides	Ligand of G-protein coupled receptor	[120,121]	Type 2 Diabetes Mellitus, dietary supplementation, phase 1	NCT01453842

Function of metabolites is highly context-dependent making therapeutic use challenging

Wang, Z. et al. *Cell*, **2015**, *163*, 1585

Descamps, H. C.; Herrman, B.; Wiredu, D.; Thaiss, C. A. *EBioMedicine*, **2019**, *44*, 747

Heterologous and Autologous Fecal Microbiota Transplantation

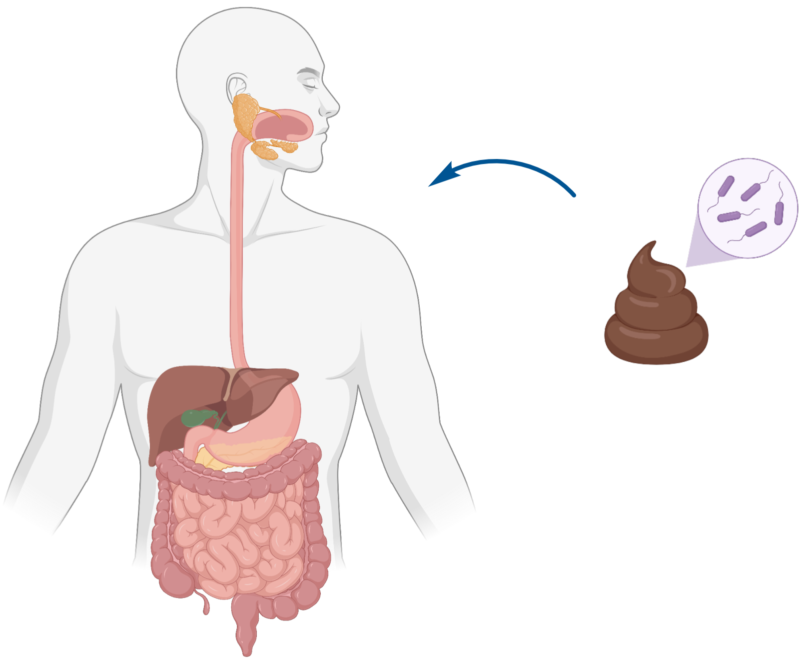
Fecal Microbiota Tranplantation

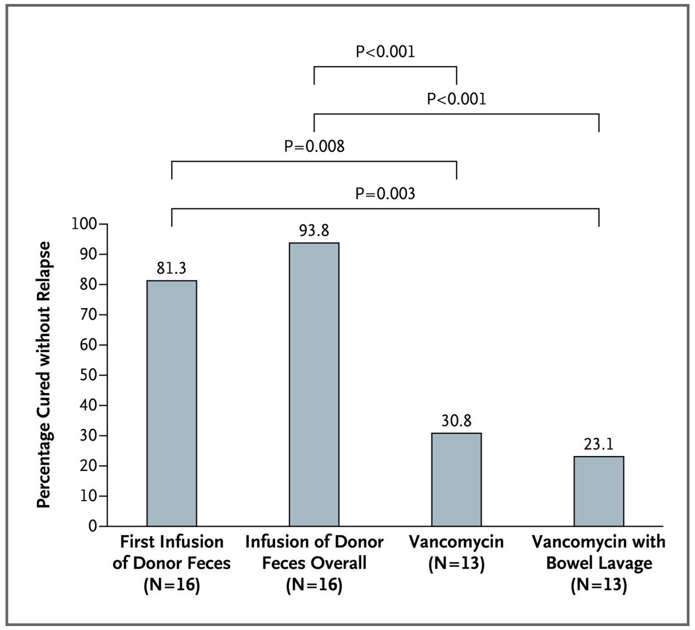
↓

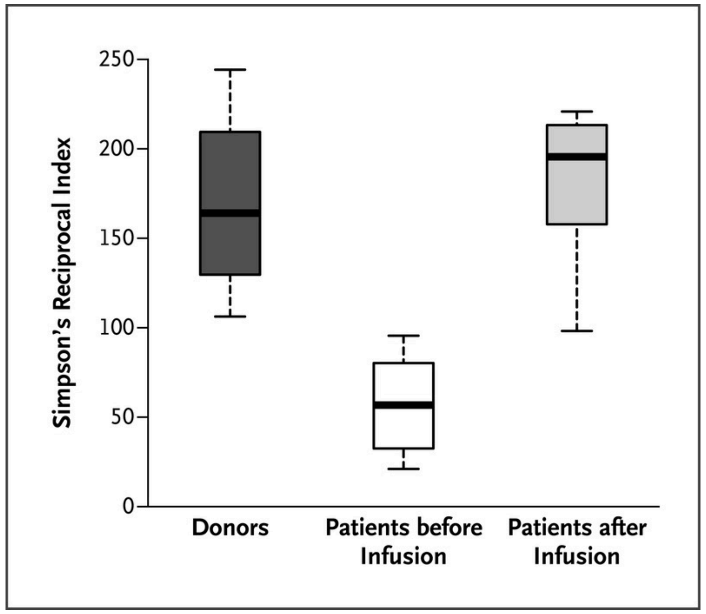
Stool or stool microbiota is transplanted into patient

Autologous = use of own feces from a healthier state

Heterologous = using feces from a healthy donor







For recurrent C. difficile infection, FT is the only true effective treatment!

Nood, E.; Vrieze, A.; Nieuwdorp, M.; Fuentes, S. Et al. *N. Engl. J. Med.* **2013**, *368*, 407

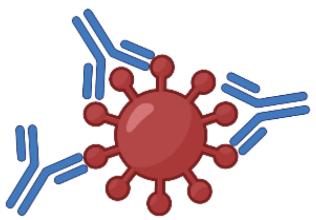
Heterologous and Autologous Fecal Microbiota Transplantation

Unknown whether heterologous FMT will be an option in preventing or treating more complex diseases

Potential indications examples:

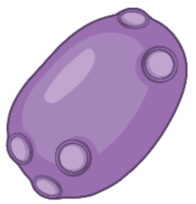
IBD, IBS, Crohn's, T2D, Obesity, Autoimmune Disorders, Parkinson's

Multiple challenges for heterologous FMT:



Immunological compatibility between donor and recipient

Importance of dieting for stool survival unknown



Role of bacteriophages/fungi for successful FMT unknown

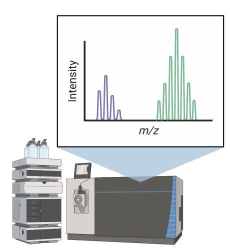
Autologous FMT would be much less complicated, but feasibility and efficacy unknown

Pedersen, O.; Fan, Y. *Nat. Rev. Microbiol.* **2021**, *19*, 55

Conclusions

This research field is still young both in its basic and translational directions

Little of the novel knowledge has been validated or has matured to a stage where it can guide public health or clinical practice



Need to find the hundreds of unknown microbial-derived chemical compounds, and investigate their significance

Incomplete microbial genome databases and lack of functional annotation for most microbial genes makes interpretation of metabolome profiling challenging

Pedersen, O.; Fan, Y. *Nat. Rev. Microbiol.* **2021**, *19*, 55

Questions?

Gut microbiota in human metabolic health and disease

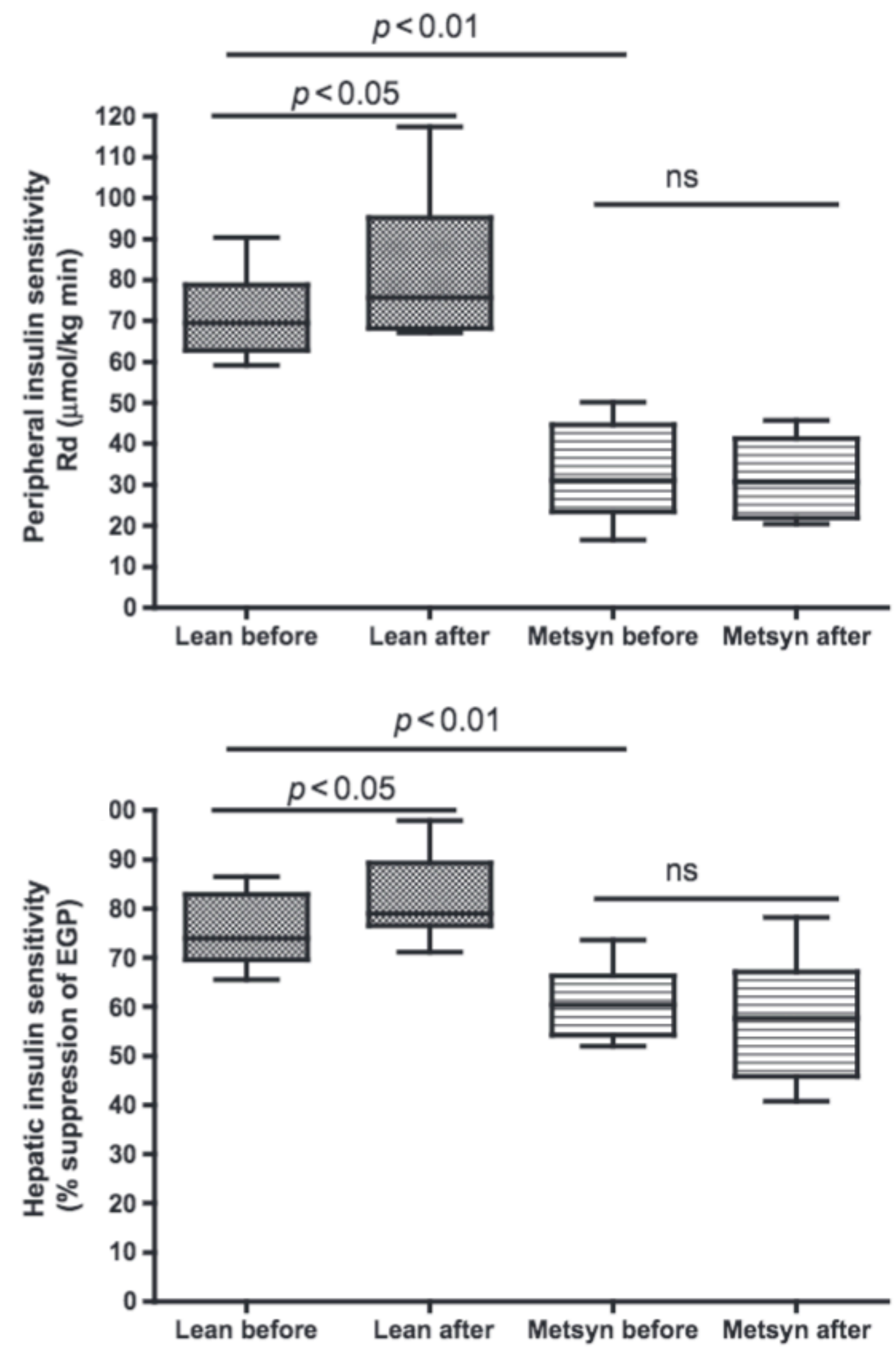
Pedersen, O.; Fan, Y. *Nat. Rev. Microbiol.* **2021**, *19*, 55

Figures created with BioRender.com

Case Study: SCFAs and Energy Homeostasis and Body Adiposity

Glucose metabolism more responsive to butyrate supplementation in lean individuals than those with metabolic syndrome

Need more studies testing combinations and different concentrations of SCFAs



Bouter, K. E. C. et al *Clin. Trans. Gastroentretol.* **2018**, *9*, 155