Evidencias actuales del papel del microbioma en la Esteatosis Hepática Metabólica: ¿puede ser una diana terapéutica?

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Conflict of interest

DISCLOSURES

- Consulting for: Alpha-sigma, Allergan, BMS, Boehringer-Ingelheim, Intercept, Innventia, Julius Clinical; Kaleido, MSD, Pfizer, Prosciento, Rubió, Shionogi, Siemens, Sobi, Thera, Zydus.
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Opinions expressed here are solely based on my own personal academic view and are intended to stimulate intelectual debate and not in any direct or indirect way drug prescription, clinical trial enrollment or any investment action.

Microbiota

conjunto de microbios patógenicos y no patógenicos (bacterias, hongos, virus y otros microorganismos como archaeas) presentes en el microbioma.

Diversidad-a

Diversidad en la taxonomía microbiana

Diversidad-B

diferencias en la composición del microbioma entre pacientes

Animal **gnotobiotico**

animal (gnotobiotico) es un animal en el que todos los microorganismos interactuando con el individuo están controlados

Microbioma

no solo se refiere a los microorganismos involucrados sino que también engloba su actividad (nicho).

Diversidad de microbioma

Medida del número (y de la distribución) de microorganismos/genes en un individuo

Catacterización taxonómica kingdom, division, class, family, genus, species and strain divisions

Metagenonoma

Análisis genético de una muestra o un entorno.

Meatgenoma-GWAS

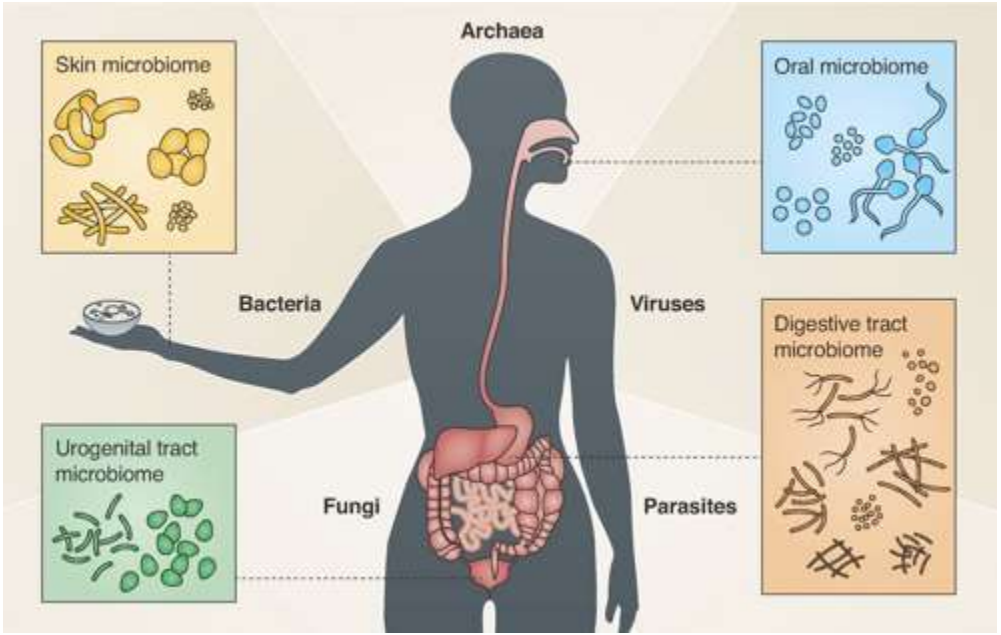
Estudios de metagenoma comepleto: Analisis de metagenoma y GWAS

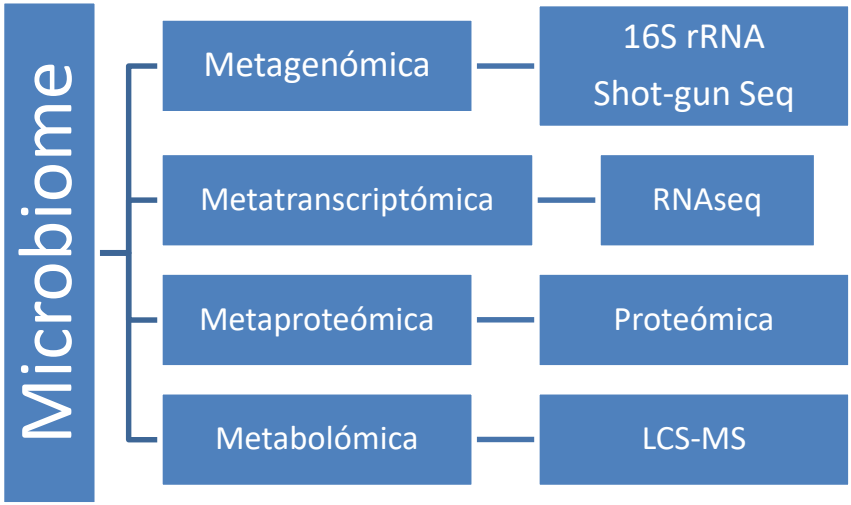
Taxonomía microbiana

algae (120), bacteria (14400), **fungi** (20200), **yeast** (4300), **protozoa** (1090), animal **viruses** (1350), **plant viruses** (590), and bacterial viruses (400).

Carmody RN et al. Nat Rev Microbiol 2023

PRINCIPALES NICHOS DE LA MICROBIOTA HUMANOS

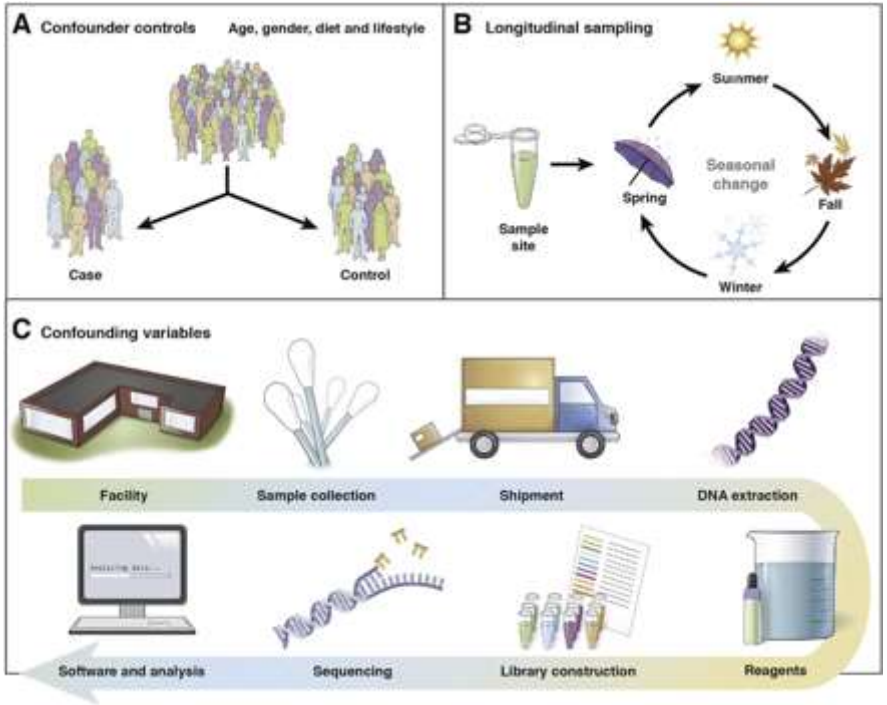


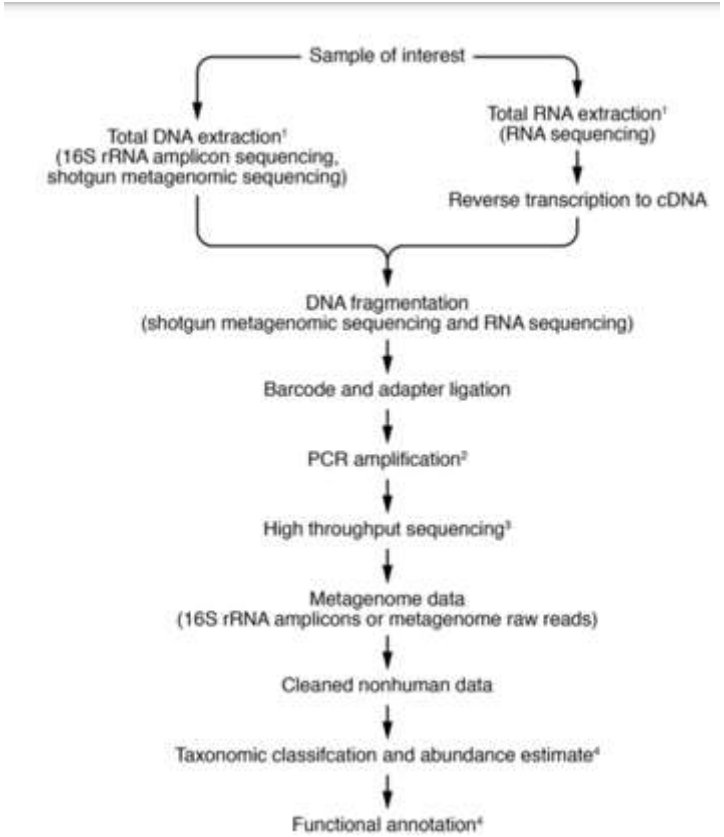


Tilg et al. Nat Metab 2021;
Wensel C et al. JCI 2022;132:154944

ÁNALISIS DEL MICROBIOMA

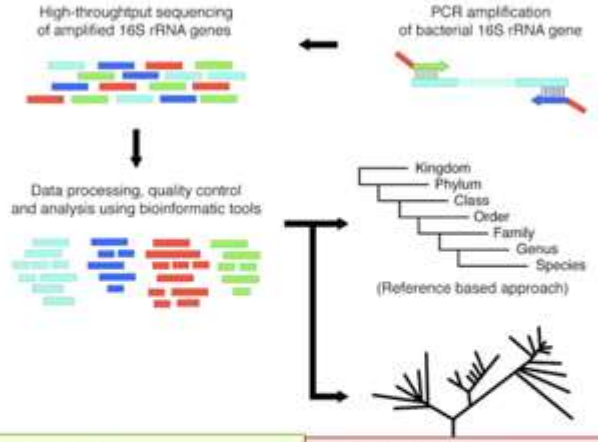
TIPO DE ÁNALISIS: COMPARACIÓN, CONFIRMACIÓN, IDENTIFICACIÓN
Nº DE MUESTRAS NECESARIAS: HUMANOS >100
ALMACENAMIENTO: (-80ºC)
SEGURIDAD: CONTAMINACIÓN DNA HUMANO





Wensel C et al. JCI 2022;132:154944

TÉCNICAS ESTUDIO DEL MICROBIOMA: 3. SECUENCIACIÓN DEL rDNA 16s

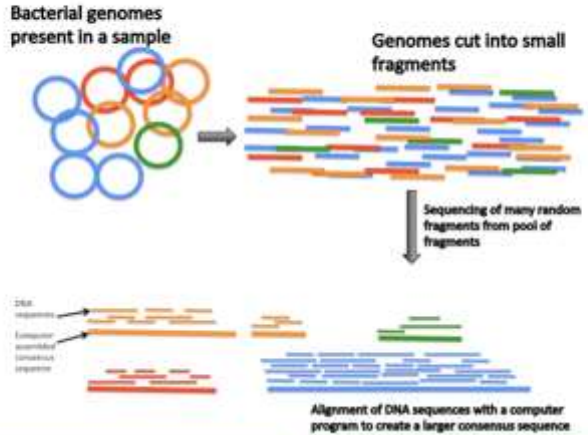


VENTAJAS
1. SENCILLO Y BIEN CARACTERIZADO
2. ASEQUIBLE
3. RÁPIDO

DESVENTAJAS
1. BAJA ESPECIFICIDAD (GÉNERO)
2. NO IDENTIFICACIÓN DE NUEVAS ESTIRPES

ÁNALISIS DE POBLACIONES BACTERIANAS DE AMPLIO ESPECTRO CON GENOMA BIEN CARACTERIZADO

TÉCNICAS ESTUDIO DEL MICROBIOMA: 4. SHOTGUN SEQUENCING



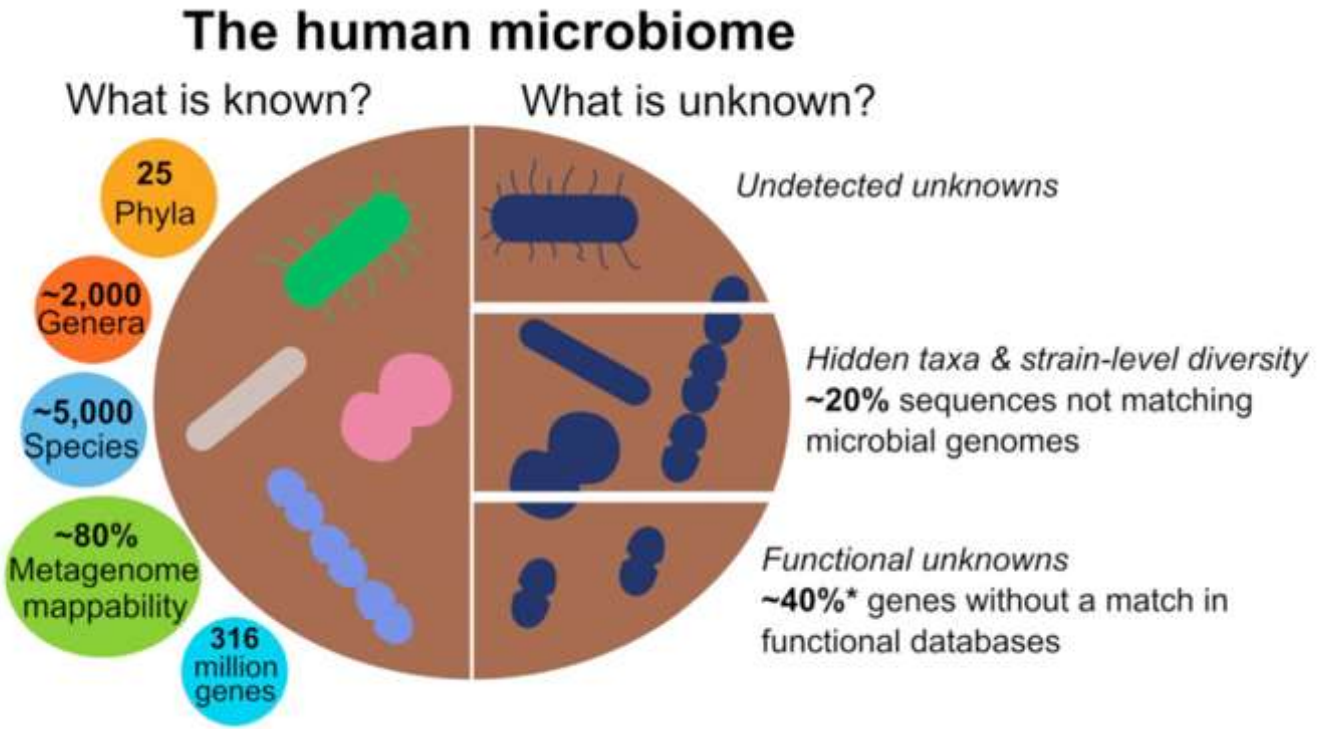
VENTAJAS
1. ESPECÍFICO (ESPECIE)
2. CUANTITATIVO
3. SENSIBLE (ng)

DESVENTAJAS
1. ALINEAMIENTO DE SECUENCIAS
2. ESPECIES NO DESCRITAS
3. CARO

CÁRACTERIZACION GLOBAL Y CUANTITATIVA DEL MICROBIOMA

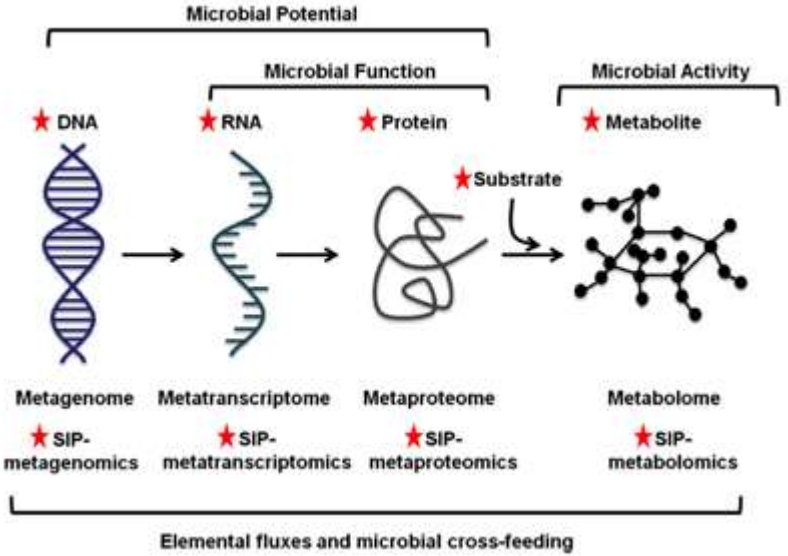
Wensel C et al. JCI 2022; 132:154944

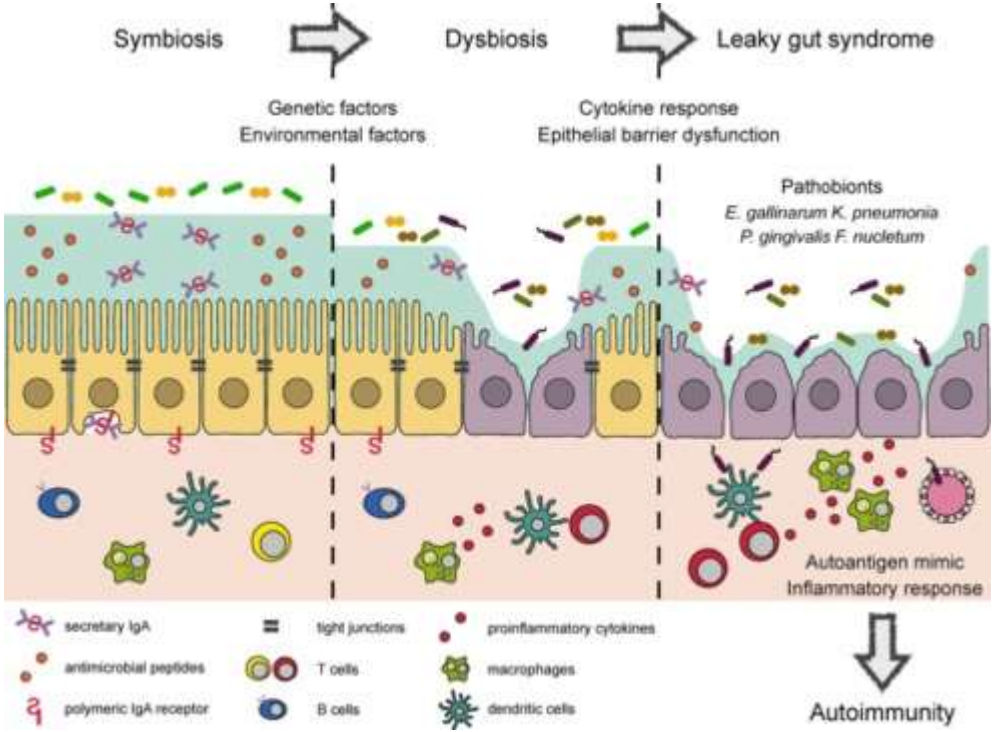
¿De qué hablamos cuando hablamos de microbiota?



Thomas & Segata, BMC Biol 2019

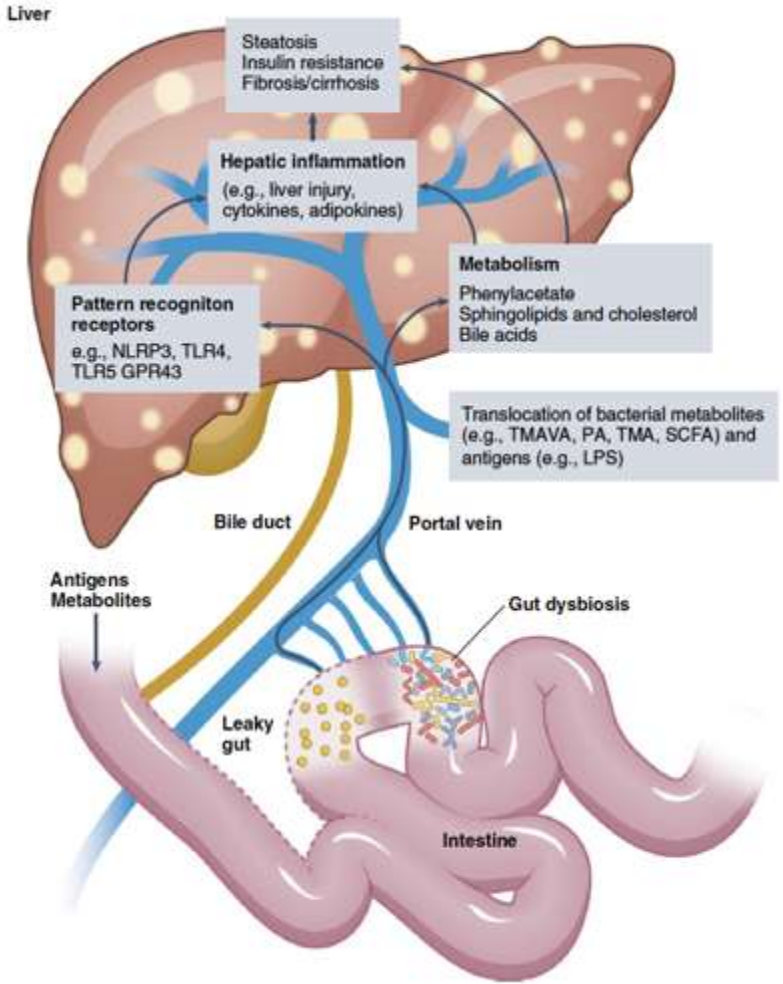
GUT-LIVER AXIS
MICROBIOME >> INTESTINAL & VASCULAR BARRIER >> LIVER SINUSOIDS

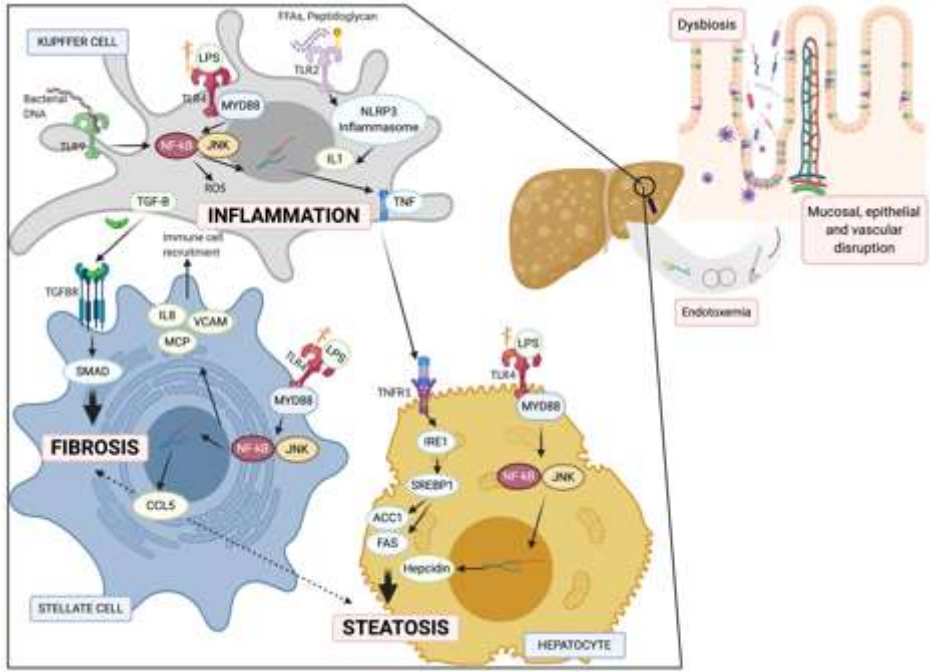




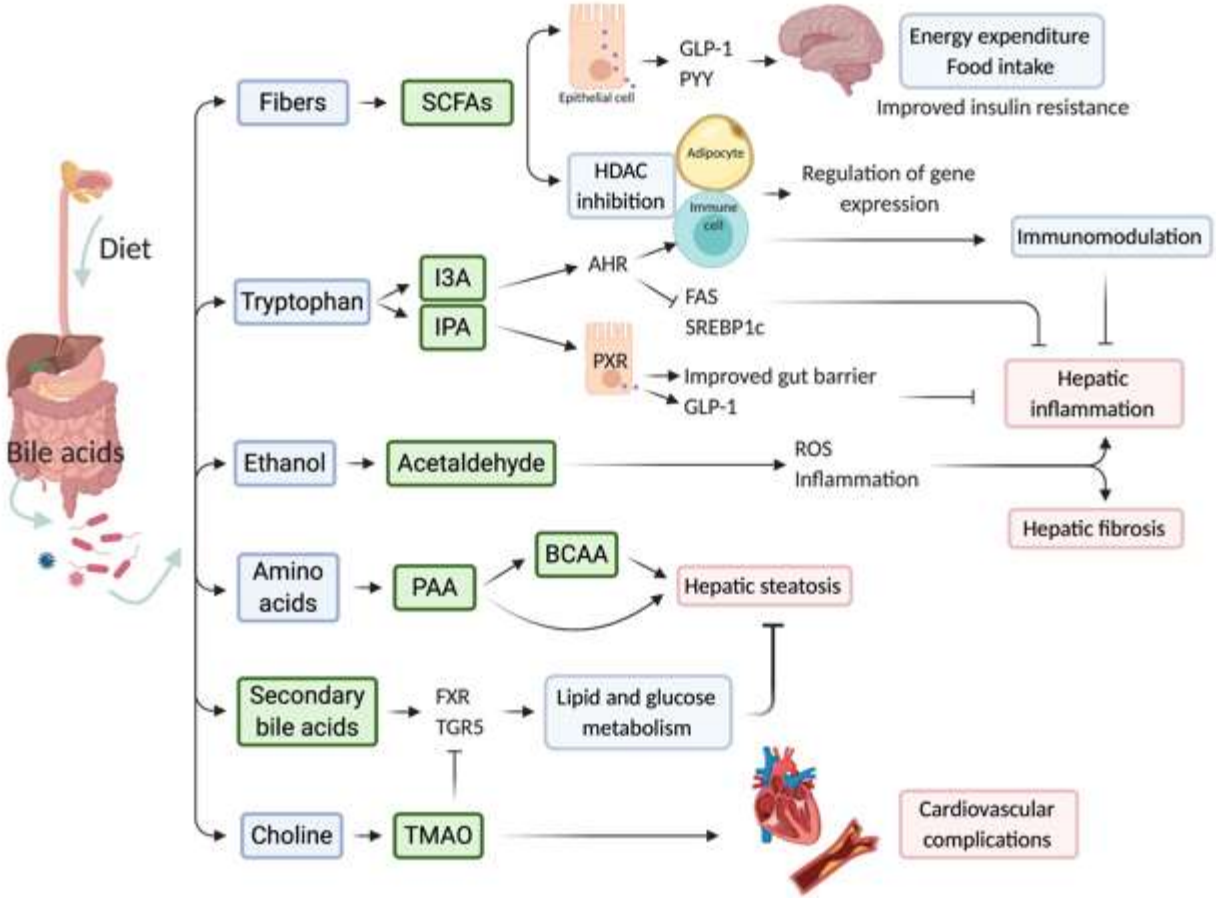
Kinashi et al., *Front Immunol* 2021; Gil-Gómez et al., 2021

PAPEL DE LA MICROBIOTA EN LA PROGRESIÓN DE LA FIBROSIS HEPÁTICA

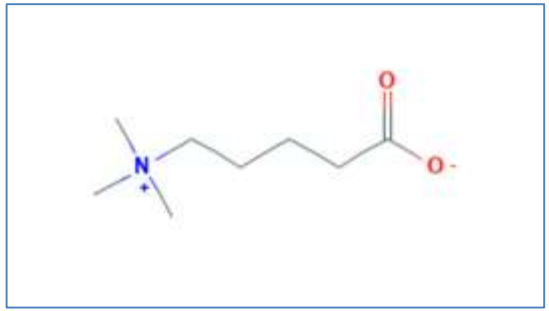




Tilg et al. Nat Metab 2021; 3:1596
Kinashi et al., *Front Immunol* 2021; Gil-Gómez et al., 2021

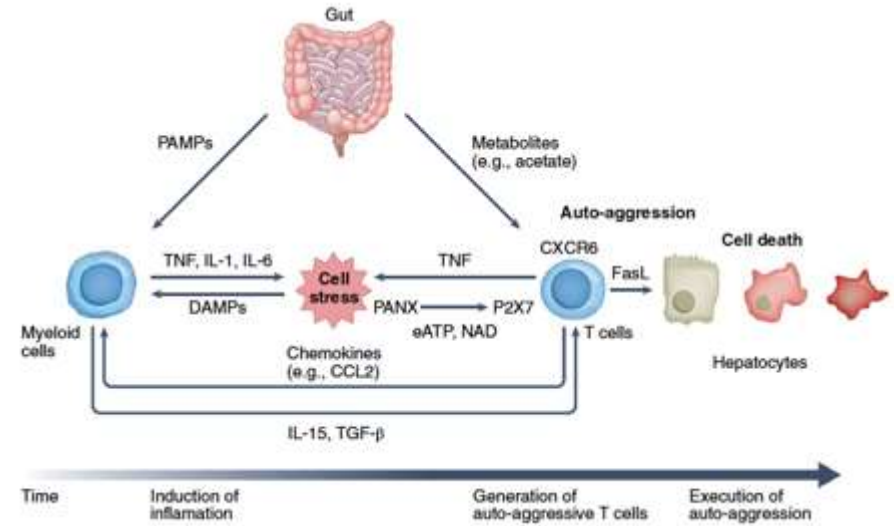


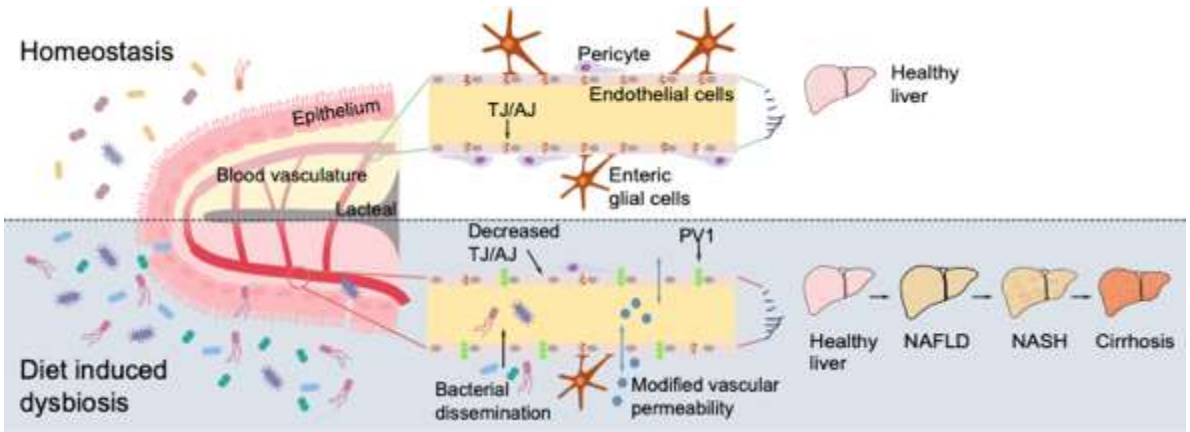
Delta-valerobetaine

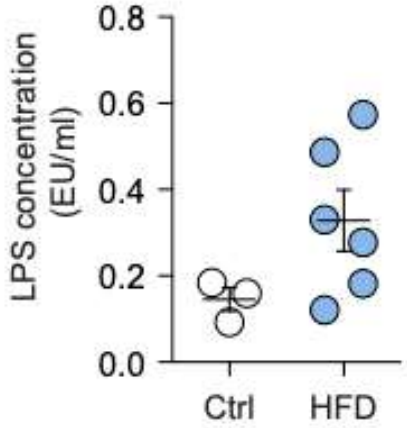


Gil-Gómez et al., *Semin Liver Dis* 2021

Microbiota-driven gut vascular barrier disruption is a prerequisite for fibrosis progression on MAFLD: role of immune-mediated inflammatory response

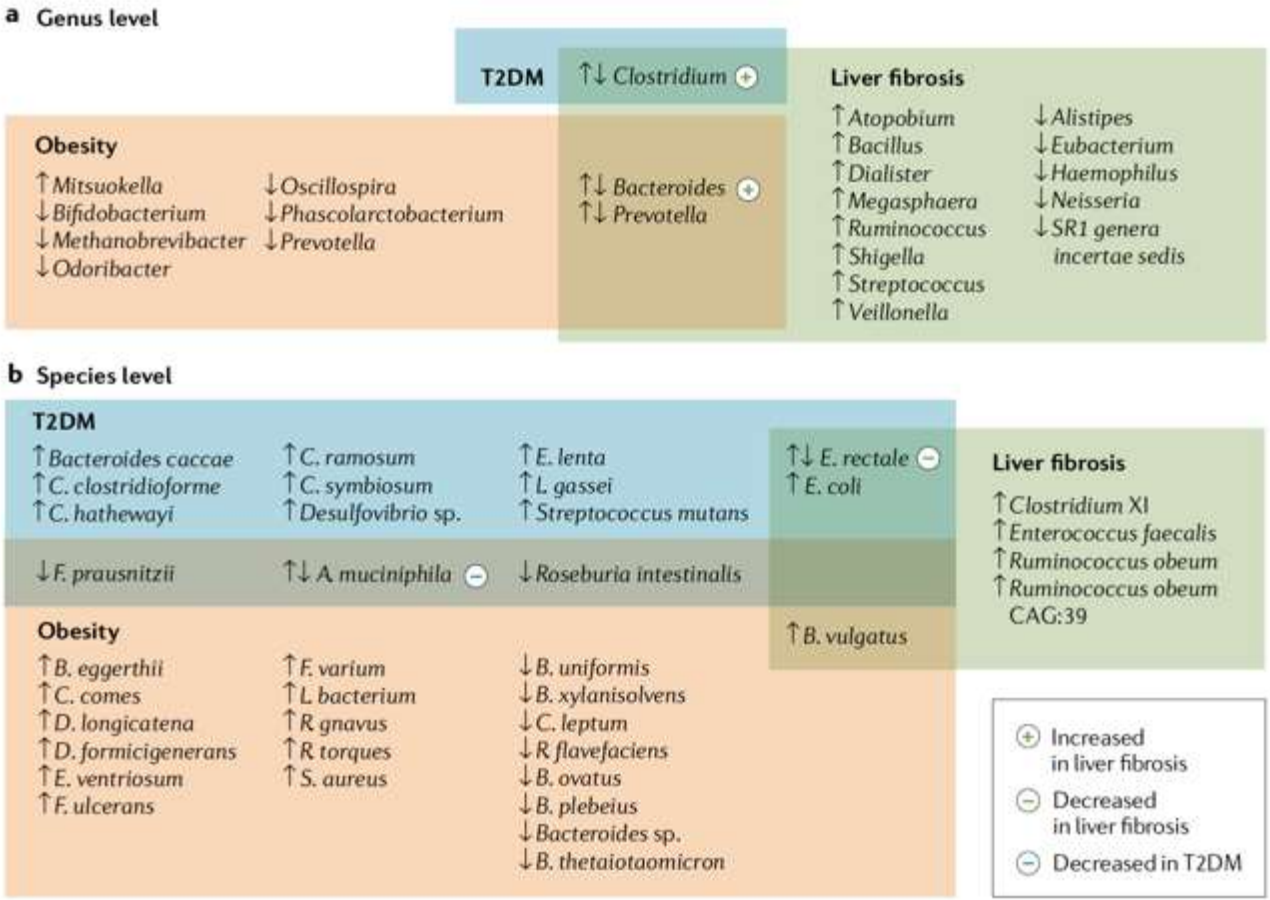






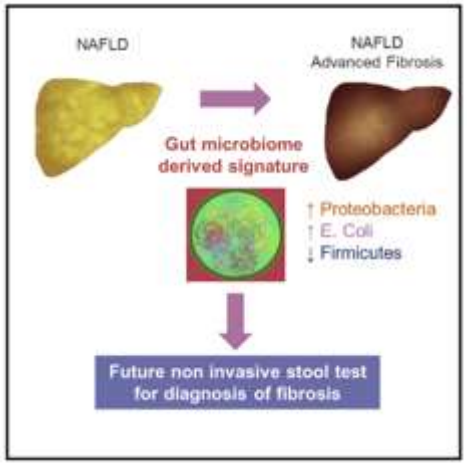
Mouries et al., *J Hepatol* 2019
Tilg et al. Nat Metab 2021; 3:1596

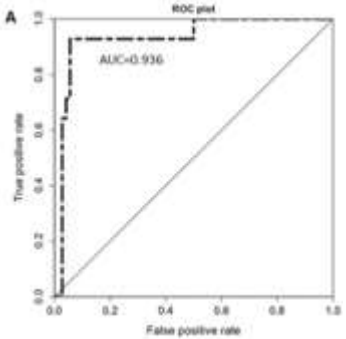
Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders

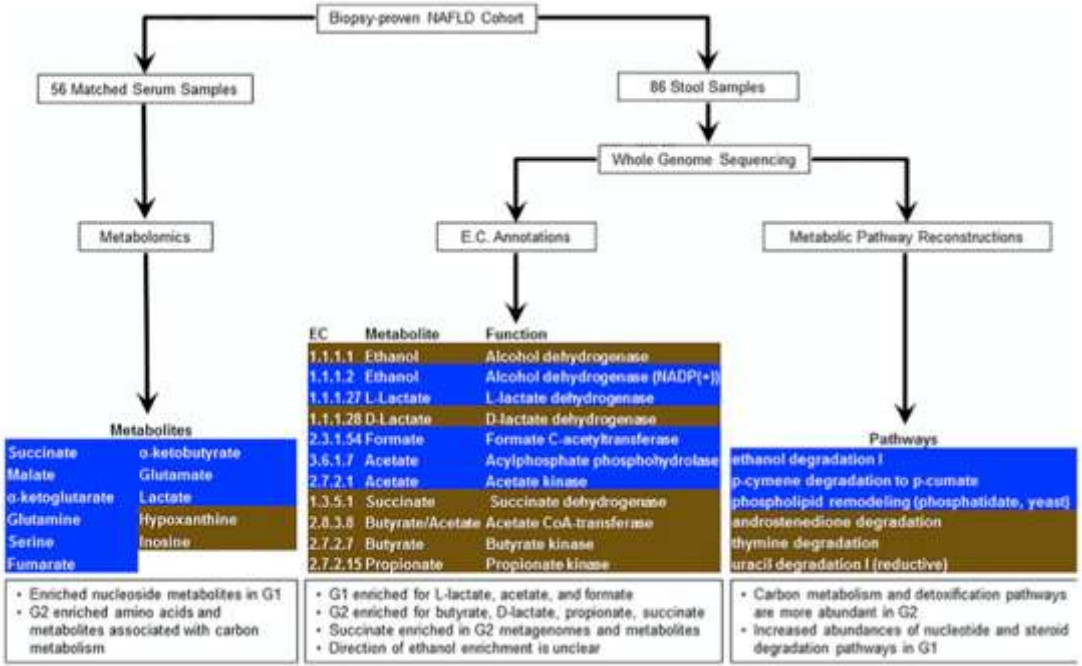


Aron-Wisnewsky J et al. NRGH 2020; 17:219

Gut Microbiome-Based Metagenomic Signature for Non-invasive Detection of Advanced Fibrosis in Human Nonalcoholic Fatty Liver Disease

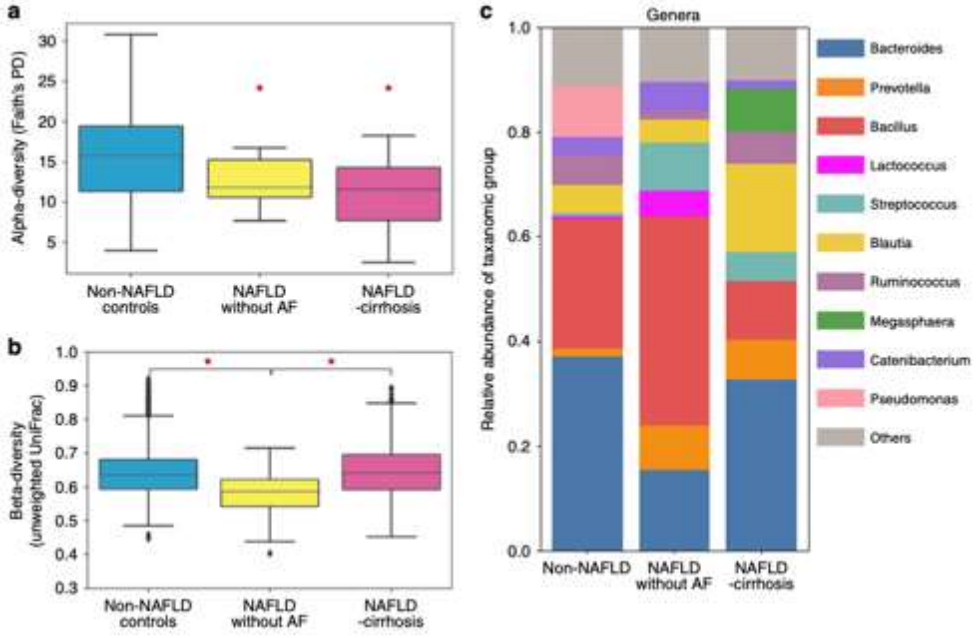


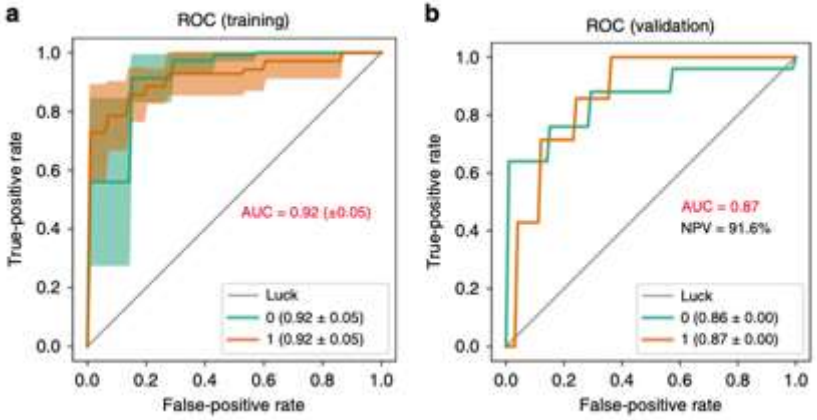




Loomba, *Cell Metab* 2017

Gut microbiome signature for cirrhosis due to nonalcoholic fatty liver disease

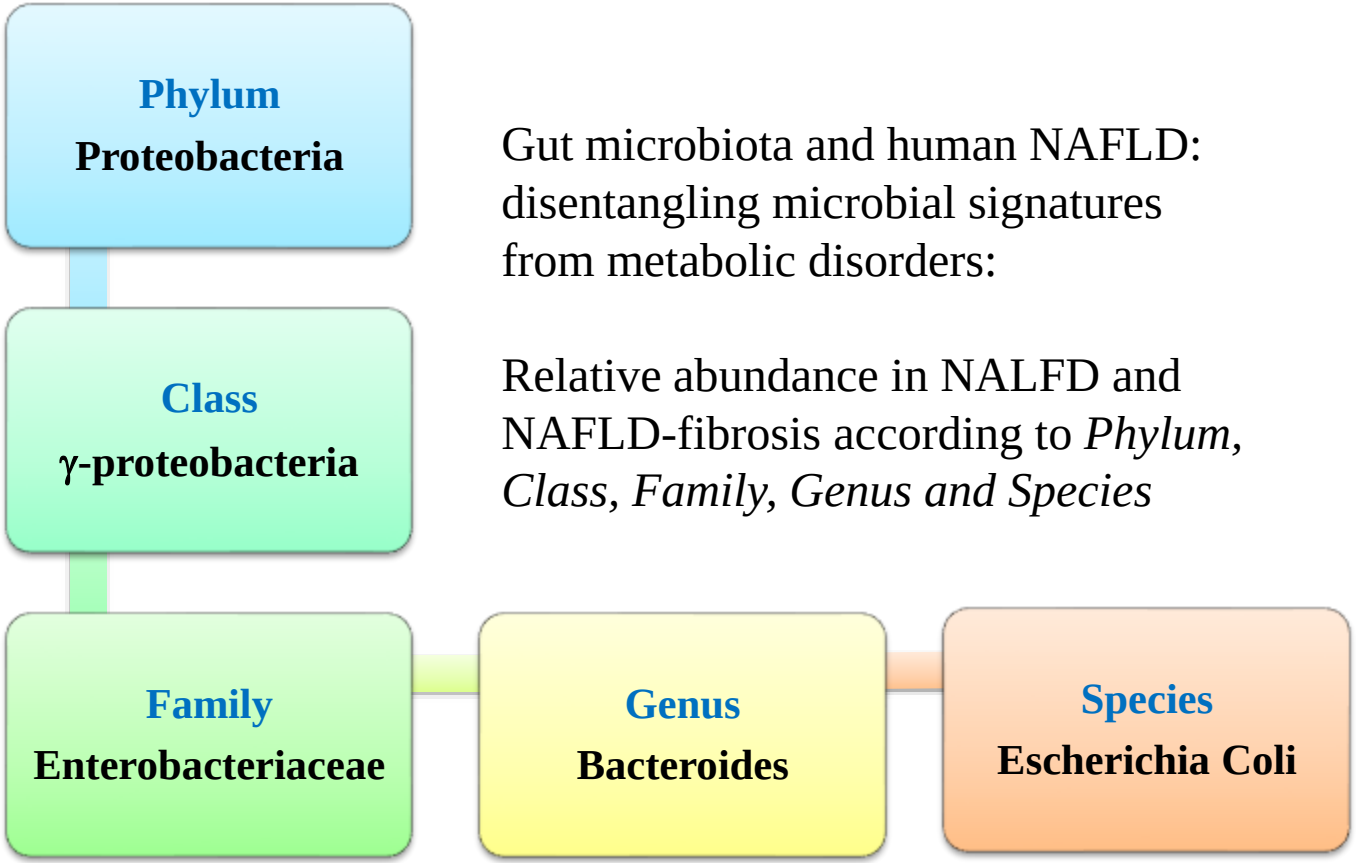




High diagnostic accuracy of a gut-microbiome signature for the detection of NAFLD-cirrhosis

Consistent with preclinical studies, these studies indicate an association between gram-negative bacteria and progression of liver fibrosis

Caussy, *Nat Commun* 2019

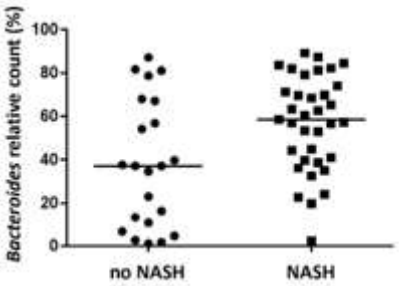


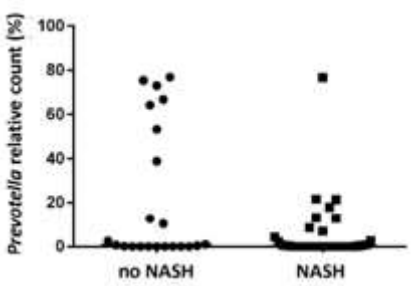
Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders:

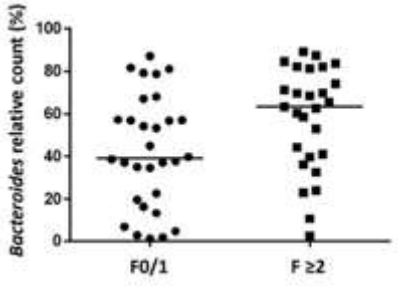
Relative abundance in NALFD and NAFLD-fibrosis according to *Phylum, Class, Family, Genus and Species*

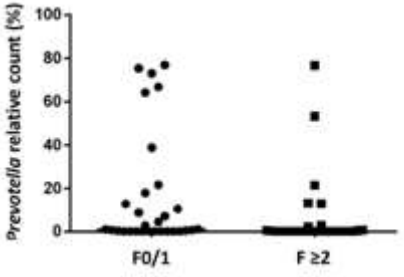
Aron-Wisnewsky J et al. NRGH 2020; 17:219

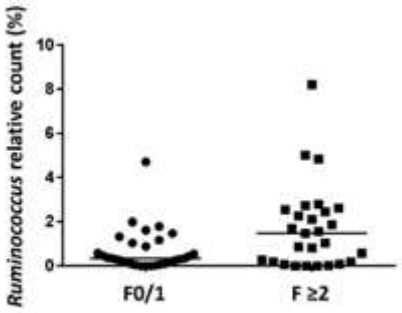
Abundance of *Ruminococcus* –> sig. fibrosis

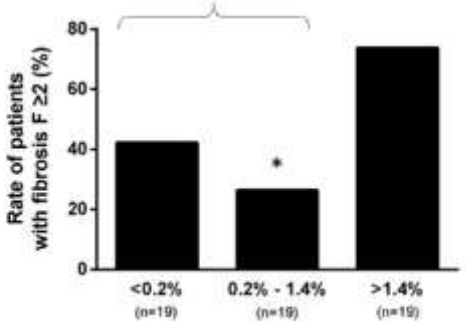












Bacteria	F0/F1 (n = 30)	F≥2 (n = 27)	*P* Value*
Actinobacteria	**0.9**	**1.8**	**0.987**
Bifidobacteriaceae	0.9	1.8	0.949
Bifidobacterium	*0.9*	*1.8*	*0.949*
Bacteroidetes	**66.2**	**69.6**	**0.388**
Bacteroidaceae	42.4	57.8	0.018
Bacteroides	*42.4*	*57.8*	*0.018*
Porphyromonadaceae	1.9	1.0	0.231
Parabacteroides	*1.9*	*1.0*	*0.231*
Prevotellaceae	16.2	6.8	0.017
Prevotella	*16.2*	*6.8*	*0.017*
Rikenellaceae	2.0	1.6	0.949
Paraprevotellaceae	2.8	0.8	0.386
Firmicutes	**26.7**	**25.4**	**0.798**
Clostridiales; unknown†	1.7	1.4	0.270
Lachnospiraceae	10.9	11.3	0.774
Blautia	*1.9*	*1.6*	*0.975*
Unknown†	*4.9*	*5.9*	*0.397*
Ruminococcaceae	8.6	7.5	0.576
Ruminococcus	*0.7*	*1.7*	*0.037*
Unknown†	*7.2*	*5.1*	*0.250*
Veillonellaceae	2.9	2.8	0.620
Megasphaera	*1.2*	*1.9*	*0.891*
Erysipelotrichaceae	1.6	0.7	0.010
Proteobacteria	**3.8**	**2.1**	**0.129**
Alcaligenaceae	1.4	0.8	0.482
Sutterella	*1.4*	*0.8*	*0.482*
Enterobacteriaceae	1.9	1.0	0.099
Unknown†	*1.5*	*0.7*	*0.128*

TABLE 4. Functional Profile of the Gut Microbiota From NASH or F≥2 Patients

KO Functional Categories Level 2	Level 3	NASH vs. No NASH LDA	NASH vs. No NASH *P* Value	F≥2 vs. F0/F1 LDA	F≥2 vs. F0/F1 *P* Value
Biosynthesis of other secondary metabolites	Phenylpropanoid biosynthesis	2.34	0.03	—	—
Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	2.28	0.03	2.28	0.02
Carbohydrate metabolism	Pentose and glucuronate interconversions	—	—	2.74	0.02
Carbohydrate metabolism	Pentose phosphate pathway	—	—	2.44	0.01
Carbohydrate metabolism	Starch and sucrose metabolism	2.68	0.01	—	—
Carbohydrate metabolism	Unclassified	2.22	0.02	2.27	0.01
Lipid metabolism	Fatty acid biosynthesis	—	—	2.10	0.03
Lipid metabolism	Lipid biosynthesis proteins	—	—	2.07	0.03
Lipid metabolism	Sphingolipid metabolism	2.45	0.04	—	—
Metabolism of other amino acids	Cyanoamino acid metabolism	2.27	0.03	—	—
Translation	Translation factors	—	—	-2.25	0.05
Replication and repair	DNA replication proteins	—	—	-2.55	0.04

Boursier, *Hepatology* 2016

Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders

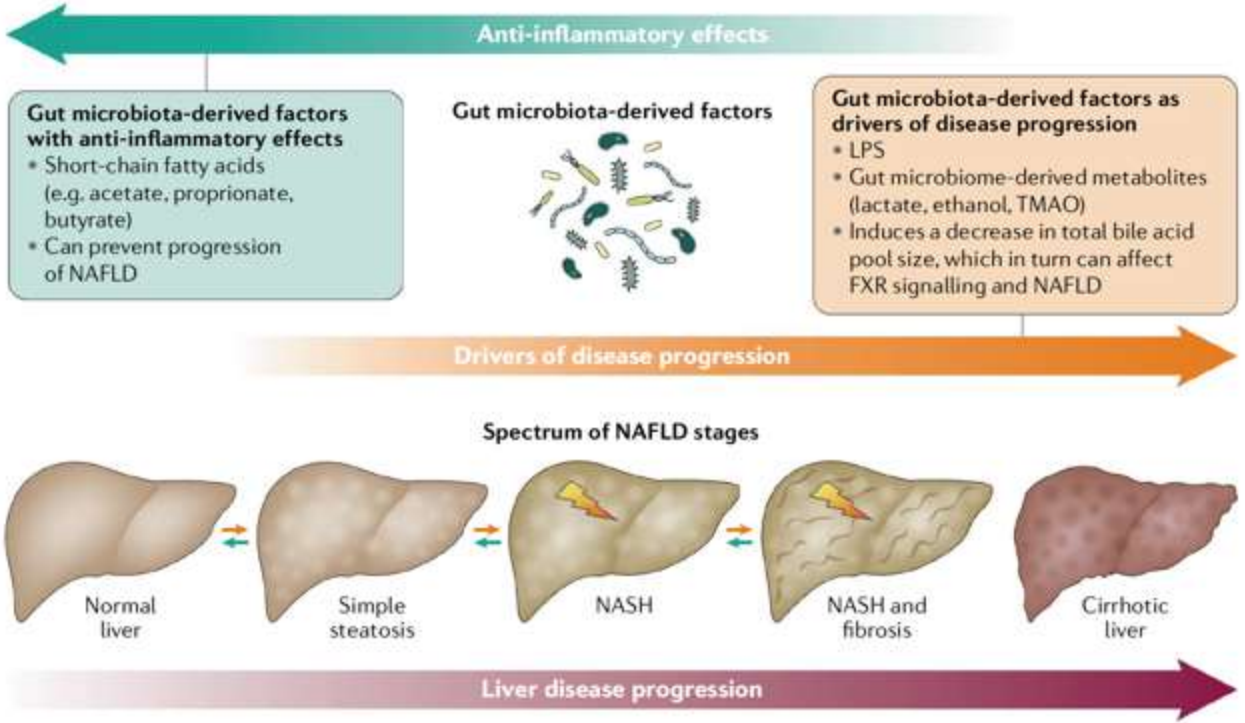
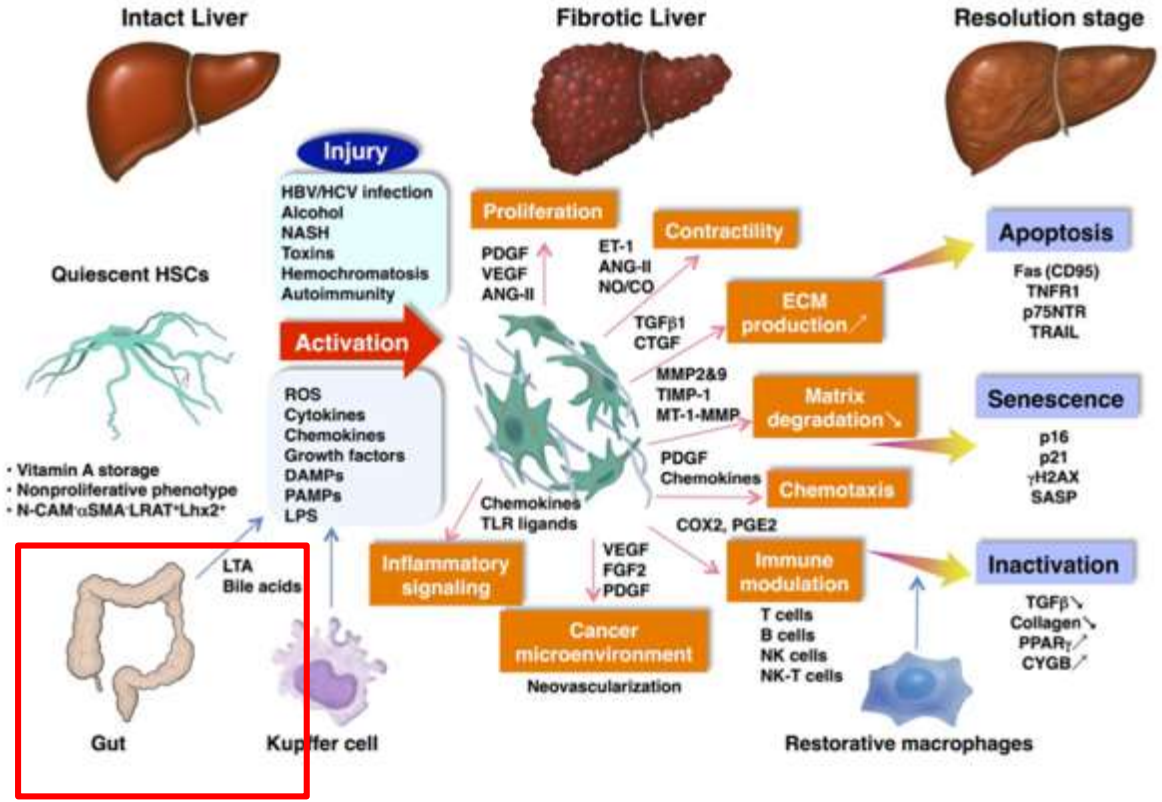


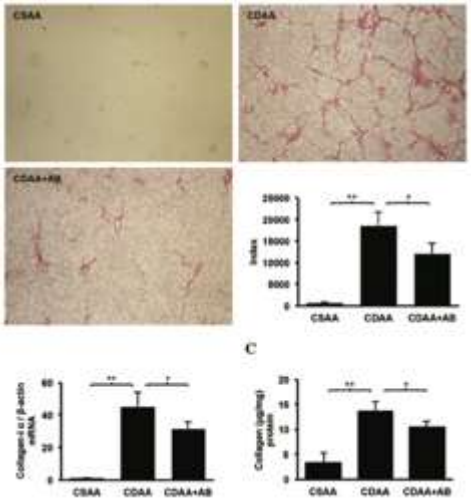
Fig. 3 | **Gut-derived metabolites and factors that could drive progression of NAFLD.** This figure illustrates how gut-derived metabolites are involved in the development and progression of non-alcoholic fatty liver disease (NAFLD), fibrosis and cirrhosis. Lactate, ethanol, trimethyl *N*-oxide (TMAO) can propel NAFLD progression (TMAO induces a decrease in the total bile acid pool size, which in turn can affect FXR signalling and NAFLD), as can lipopolysaccharide (LPS). On the other hand, short-chain fatty acids can have anti-inflammatory properties, which could prevent progression of NAFLD. NASH, non-alcoholic steatohepatitis.

Aron-Wisnewsky J et al. NRGH 2020; 17:219

Gut-derived signals induce HSCs activation



Tto con antibióticos mejora la fibrosis inducida por dieta CDAA.
KO mice para receptores de subproductos bacterianos (TLR2, TLR4, TLR9) están protegidos frente al desarrollo de fibrosis.



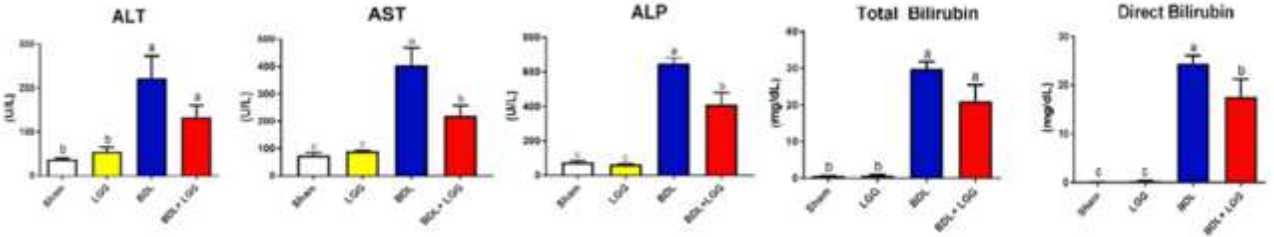
Douhara, *Mol Med Rep,* 2014; Ohtani, *Hepatol Commun* 2019

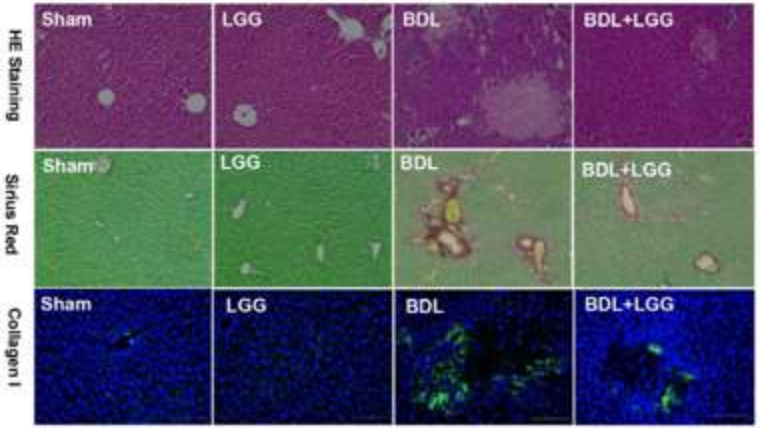
POTENCIAL TERAPÉUTICO DE LA MICROBIOTA

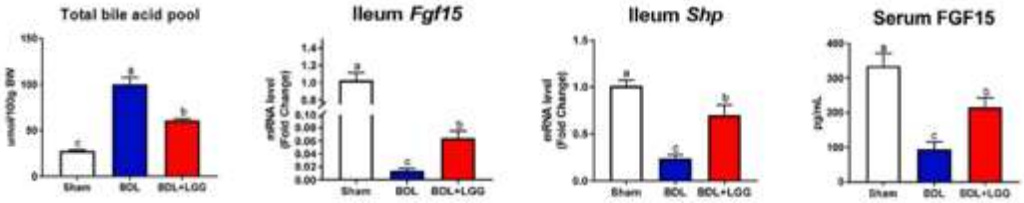
Place of action	Denomination	Principle of action
Intestinal content	Hydrogel technology	Modified cellulose cross-linked with citric acid that mimics natural dietary fibres in vegetables. Hydrogel particles rapidly absorb water in the stomach and increase in volume, thereby improving satiety. Once in the large intestine, the hydrogel is partially digested and releases water, which is then reabsorbed.
	Gut-restricted polymers	Insoluble cross-linked polymeric drugs selectively bind with high-avidity multivalent surface features on bacteria or viruses, toxins, inorganic ions including potassium, phosphate, or bile acids.
	Carbon nanoparticles	Non-absorbable carbon particles exhibit a high adsorptive capacity for bacterial toxins and represent a novel strategy to counteract dysbiosis and translocation of bacterial-derived products.
	Non-selective beta-blockers	Beta-blockers reduce the load of enteric bacteria and inhibit intestinal bacterial overgrowth by fastening intestinal transit time and reducing intestinal permeability
Intestinal microbiome	Non-absorbable antibiotics	Selectively reduce the burden of enteric bacteria that mostly contribute to translocation, *e.g.* gram-negative bacteria. Rifaximin is a broad-spectrum compound, which exerts endotoxin-lowering and anti-inflammatory effects largely independent from their bactericidal action.
	Bacteriophages	Bacteriophages are viruses that specifically infect and kill intestinal bacterial pathogens. In contrast to antibiotics, phages do not induce resistance.
	Synthetic live bacterial therapeutics	These are engineered probiotics that can selectively consume toxic metabolites in the intestine and convert them into nontoxic forms.
	Fecal microbial transplantation	Fecal microbial transplantation is a method to replenish a healthy gut microbial environment and restore physiological colonization by recolonising the intestine with microbial flora from a healthy donor.
Intestinal mucosa	Pharmacological modulation of gut peptides.	Specific agonists of mucosal gut receptors may elicit responses including the release of regulators of glucose or bile acid metabolism.
	Duodenal mucosal resurfacing	Superficial duodenal mucosal ablation mediates an abnormal signal to endogenous insulin-sensitive tissues by limiting nutrient exposure or contact with the duodenal mucosa.
	Postbiotics	Postbiotics are metabolic products from intestinal bacteria that include short-chain fatty acids, secondary bile acids, proteins polysaccharides, vitamins and organic acids acting as metabolic regulators.
	FXR agonists	FXR-agonists reconstitute microbiota composition, restore epithelial and vascular intestinal barrier function, improve intestinal innate defense mechanisms, reduce intestinal inflammation and decrease bacterial translocation and endotoxemia
Peritoneal cavity	Ammonia uptake particles	This treatment consists in a suspension of large transmembrane pH-gradient liposomes, containing citric acid and designed to rapidly capture ammonia from ascites of cirrhotic patients.

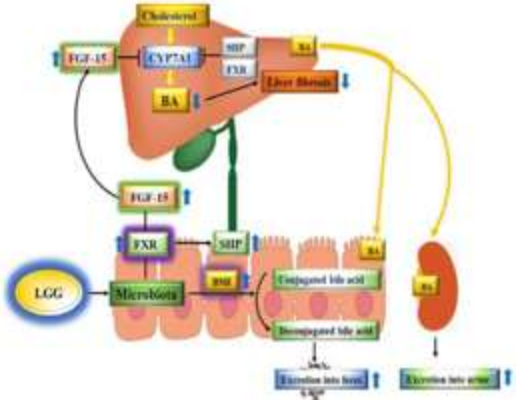
Wensel C et al. JCI 2022; 132:154944

Probiotic *Lactobacillus rhamnosus* GG Prevents Liver Fibrosis Through Inhibiting Hepatic Bile Acid Synthesis and Enhancing Bile Acid Excretion in Mice

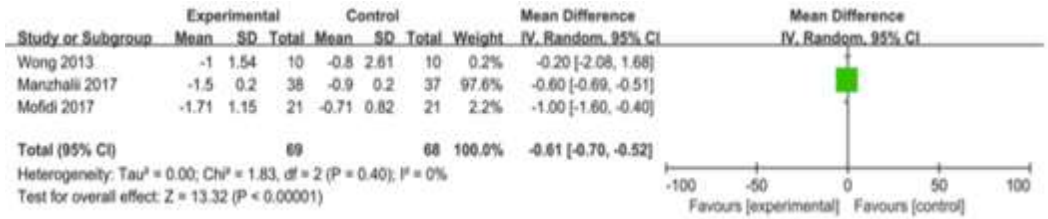








Liu, *Hepatology* 2020



Metaanálisis: **probióticos** mejoran liver stiffness

	Probiotics (n = 17)		Placebo (n = 22)		
	Mean	SD	Mean	SD	p-Value
Steatosis, dB/m	−21.7	42.60	−10.72	46.64	0.45
Liver stiffness, kPa	−0.25	1.77	−0.62	2.37	0.59
ALT, IU/L	14.0	50.04	1.13	26.39	0.30
AST, IU/L	2.00	17.31	−3.40	17.09	0.33
GGT, IU/L	6.2	33	1.35	28.38	0.60
Steatosis score	0.049	0.09	0.042	0.11	0.83
Fibrosis score	0.01	0.10	0.06	0.09	0.18
Activity score	−0.12	0.15	0.015	0.13	0.56
Body mass index, kg/m^2	0.7	1.46	0.82	1.06	0.81
Triglycerides, mg/dL	−0.10	0.68	−0.07	0.77	0.90
Total cholesterol, mg/dL	0.23	0.93	0.05	1.07	0.59
Fasting glucose, mg/dL	0.46	0.94	−0.44	1.31	0.03

No efecto del probiótico “MCP-BCMC®” en fibrosis

Table 3. Baseline and End-of-Study Primary Outcome Results and Regression Models Testing the Effects of the Synbiotic Intervention and Changes in *Bifidobacterium* Species on the Primary Outcomes

	Placebo		Synbiotic					
Primary outcomes	Baseline	End of study	Baseline	End of study	Difference in change from baseline to end of study[a] (95% CI), synbiotic treatment, primary analysis	Adjusted difference in change from baseline to end of study[b] (95% CI), synbiotic treatment, primary analysis	Adjusted difference in change from baseline to end of study[c] (95% CI), *Bifidobacterium* species	Difference in change in weight from baseline to end of study[a]
MRS-measured liver fat, %	22.9 (12.9 to 44.7)	21.4 (10.7 to 35.9)	26.9 (9.7 to 38.4)	23.7 (13.0 to 42.0)	2.8 (−2.2 to 7.8) P = .30	4.1 (−0.02 to 8.3) P = .05	0.1 (−1.3 to 1) P = .80	1.9 (1.4 to 2.5) P < .001
ELF score	12.5 (0.7)	12.8 (0.8)	12.5 (0.9)	12.9 (0.8)	−0.0008 (−0.2 to 0.2) P = 1.0	0.02 (−0.2 to 0.2) P = .90	0.020 (−0.08 to 0.04) P = .50	0.015 (0.001 to 0.03) P = .039
NAFLD fibrosis score	−1.2 (1.3)	−1.3 (1.3)	−1.3 (1.3)	−1.6 (1.4)	−0.03 (−0.3 to 0.2) P = .80	−0.009 (−0.3 to 0.3) P = .90	0.005 (−0.02 to 0.03) P = .60	0.046 (0.008 to 0.085) P = .027

No efecto tras el tratamiento con **simbiótico**

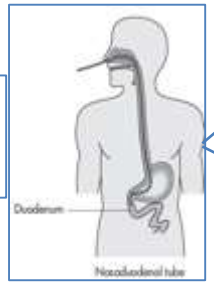
Liu, *Dig Dis Sci* 2019; Scorletti, Gastroenterology 2020; Nor, *Nutrients* 2021

HEPATOLOGY COMMUNICATIONS, VOL. 0, NO. 0, 2020

Donor Fecal Microbiota Transplantation Alters Gut Microbiota and Metabolites in Obese Individuals With Steatohepatitis

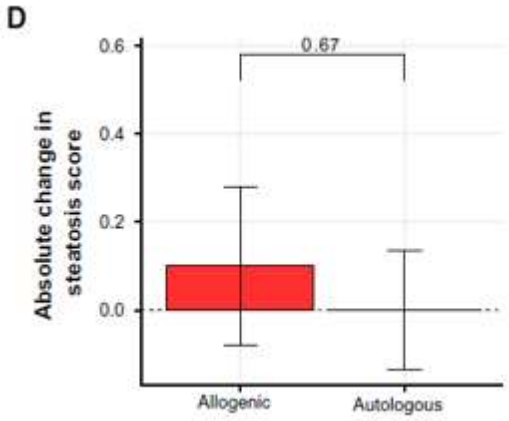
NAFLD by ultasound BMI >25 (N=21)

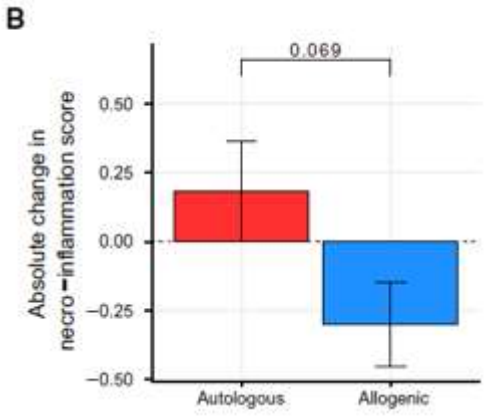
→ 3 FMT at 8 weeks interval

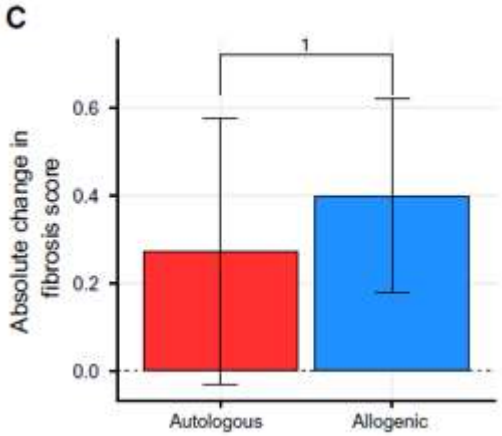


→ Autologous (N=11)

→ Allogenic (4 healthy lean vengan) (N=11)







Liver genes: increased of ARHGAP18 & SDS (serine dehydratase); and decreased RECQL5 and SF3B3
Secondary aims: GGT and ALT decreased. No difference in duodenal microbiota diversity. No significant changes in faecal microbiota diversity, but more: Ruminococcus, Eubacterium hallii, Faecalibacterium, and Prevotella copri.
Change in plasma metabolites: increased of amino acids isoleucine and phenylacetylglutamine

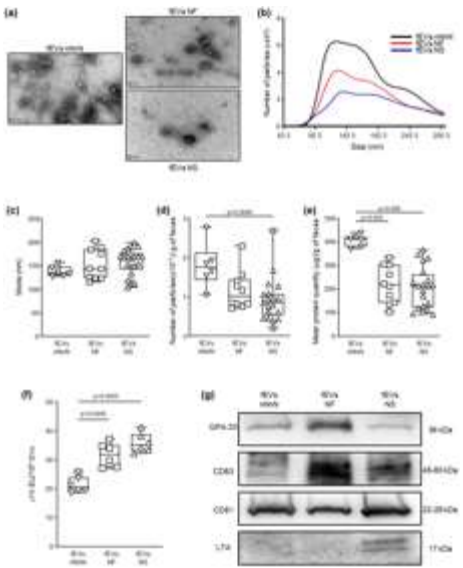
too healthy¡¡

Witjes J. Hepatology communications. 2020

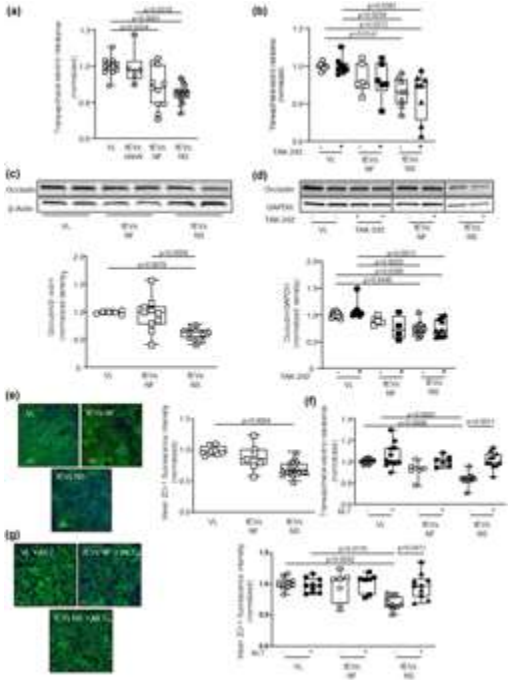
Role of EVs from faeces (fEVs) in liver diseases

- The gut microbiota participates in the progression of NAFLD towards NASH and liver fibrosis. (Bashiardes et al., 2016; Boursier & Diehl, 2016)
- Dysbiosis, a shift in bacterial community composition, has been associated with several deleterious effects in NAFLD (Boursier et al., 2016; Brandl & Schnabl, 2017).
- Dysbiosis was also reported to mediate the destabilization of intestinal and endothelial barriers leading to an increase of intestinal and endothelial permeability (Chen et al., 2015).v

EVs characterization



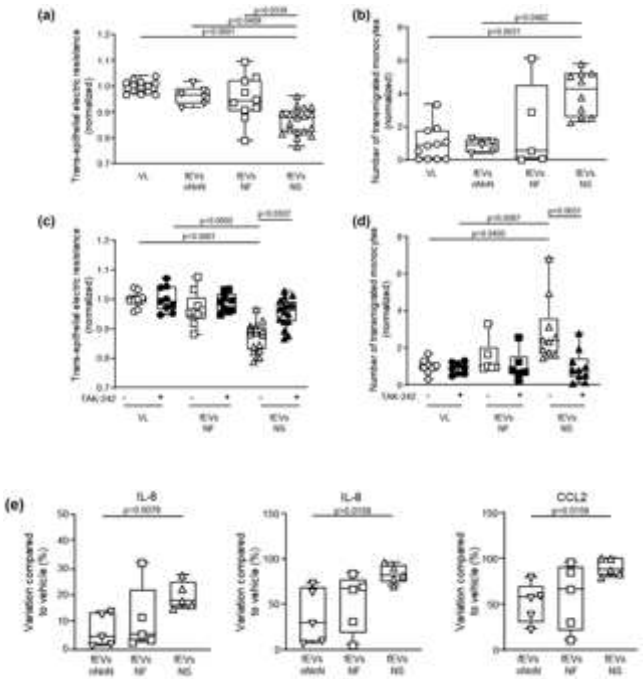
NASH fEVs enhance epithelial intestinal cell permeability and decrease occludin and ZO-1 protein expression



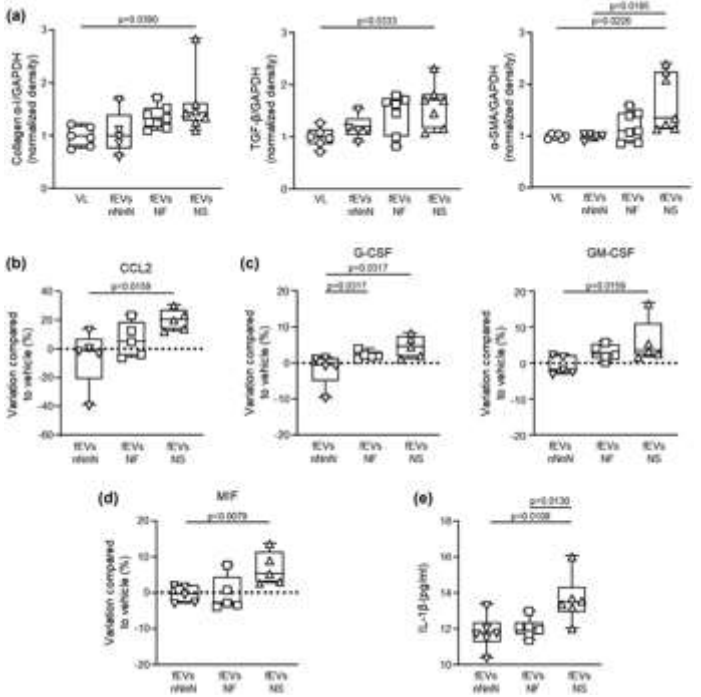
Fizanne et al. J Extracell Vesicles. 2023 Feb;12(2):e12303

Role of EVs from faeces (fEVs) in liver diseases

NASH (NS) faeces-derived extracellular vesicles (fEVs) increase endothelial permeability in a TLR4-dependent pathway and induce in vitro endothelial inflammation.



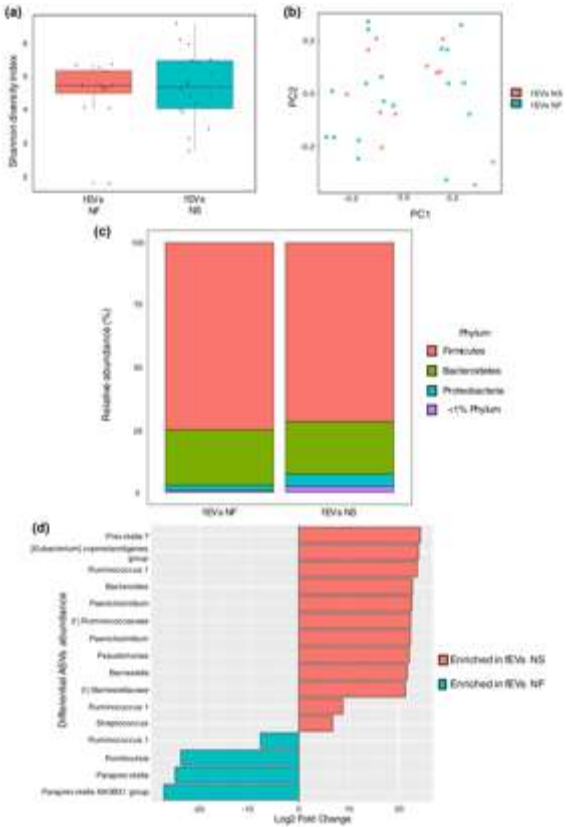
NASH (NS) faeces-derived extracellular vesicles (fEVs) activate in vitro stellate cells and increase cytokines involved in fibrogenesis



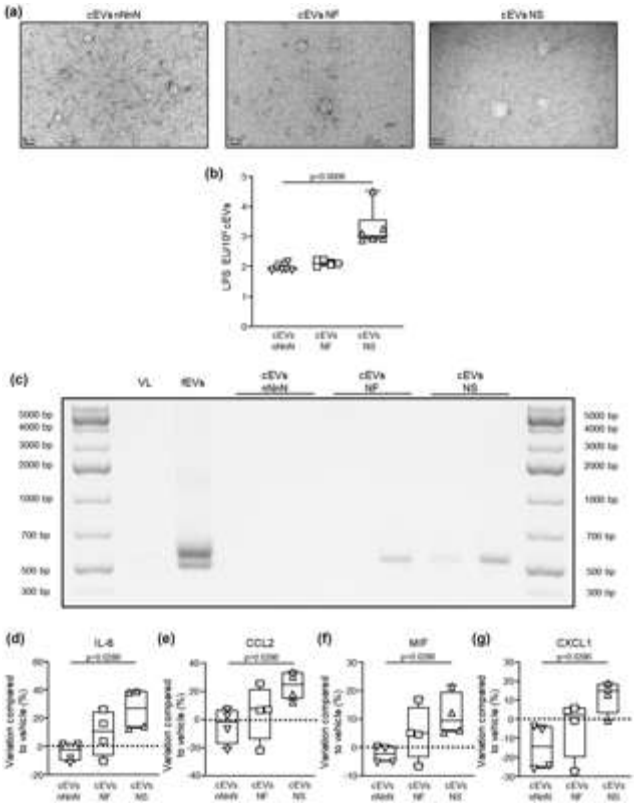
Fizanne et al. J Extracell Vesicles. 2023 Feb;12(2):e12303

Role of EVs from faeces (fEVs) in liver diseases

Metabarcoding reveals a differential composition between NAFLD and NASH fEV samples

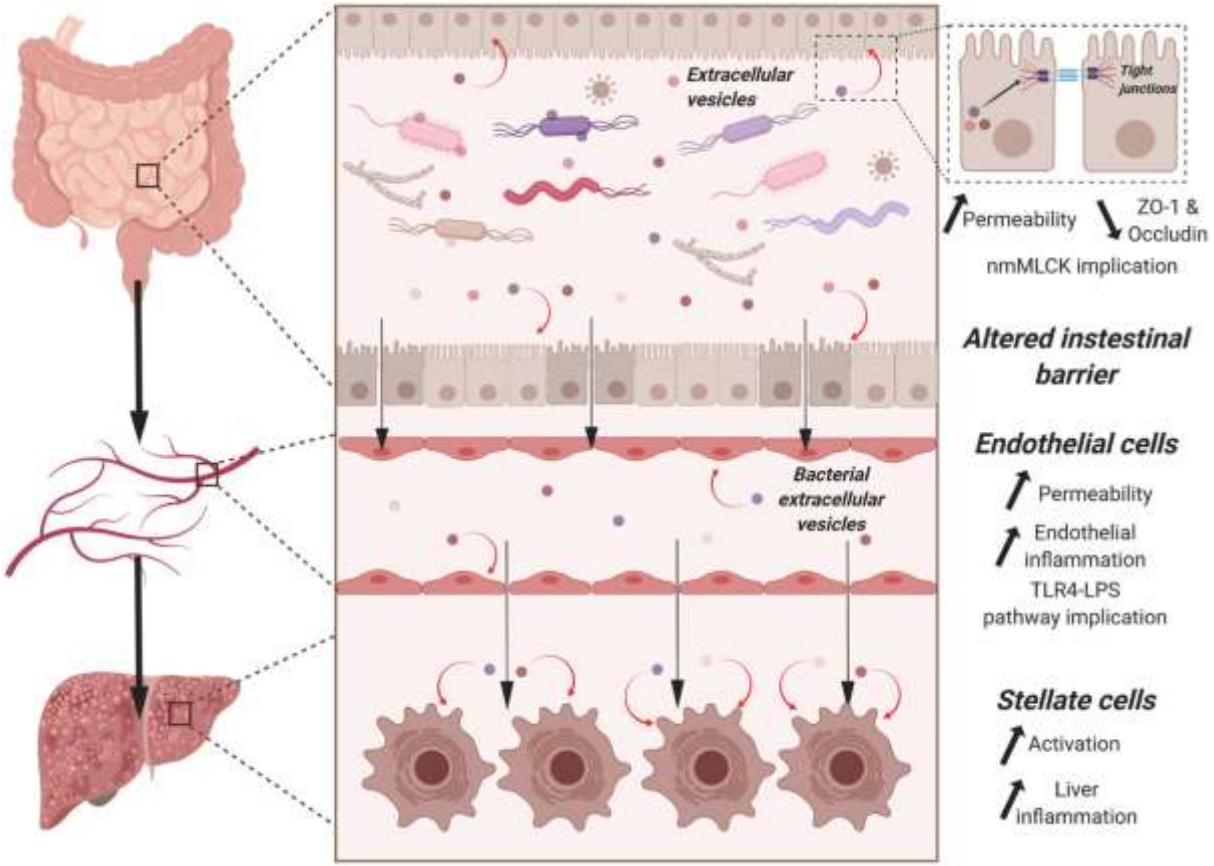


Circulating small EVs (cEVs) isolated from patients with NAFLD and NASH contained prokaryotic EVs and induced cytokine production in stellate cells



Fizanne et al. J Extracell Vesicles. 2023 Feb;12(2):e12303

fEVs are a key player in barrier dysfunction, inflammation, and liver injuries especially in patients with NASH, at the early onset of the disease.



Fizanne et al. J Extracell Vesicles. 2023 Feb;12(2):e12303

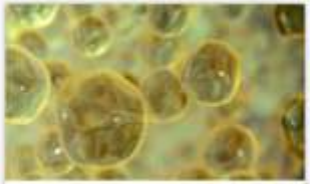
Biomimetic superabsorbent hydrogel acts as a gut protective dynamic exoskeleton improving metabolic parameters and fostering *Akkermansia muciniphila* expansion.

ORALLY ADMINISTERED

MECHANICAL PROPERTIES SIMILAR TO VEGETABLES

PROPERTIES ARE MAINTAINED THROUGH THE SMALL INTESTINE

CONTROLLED HYDRATION

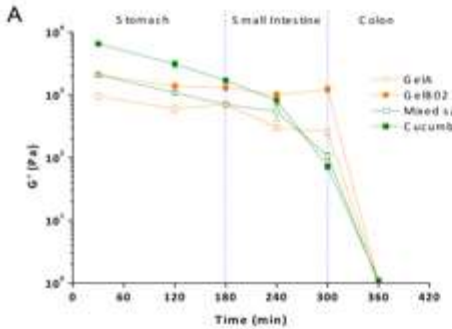


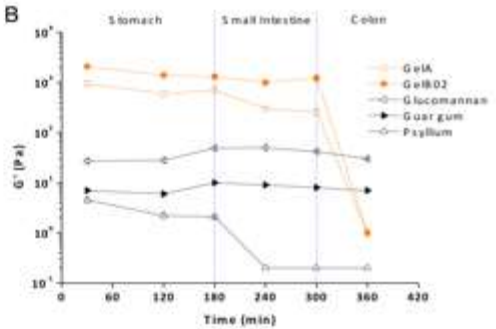
NON CLUSTERING PARTICLES

NATURAL DEGRADATION

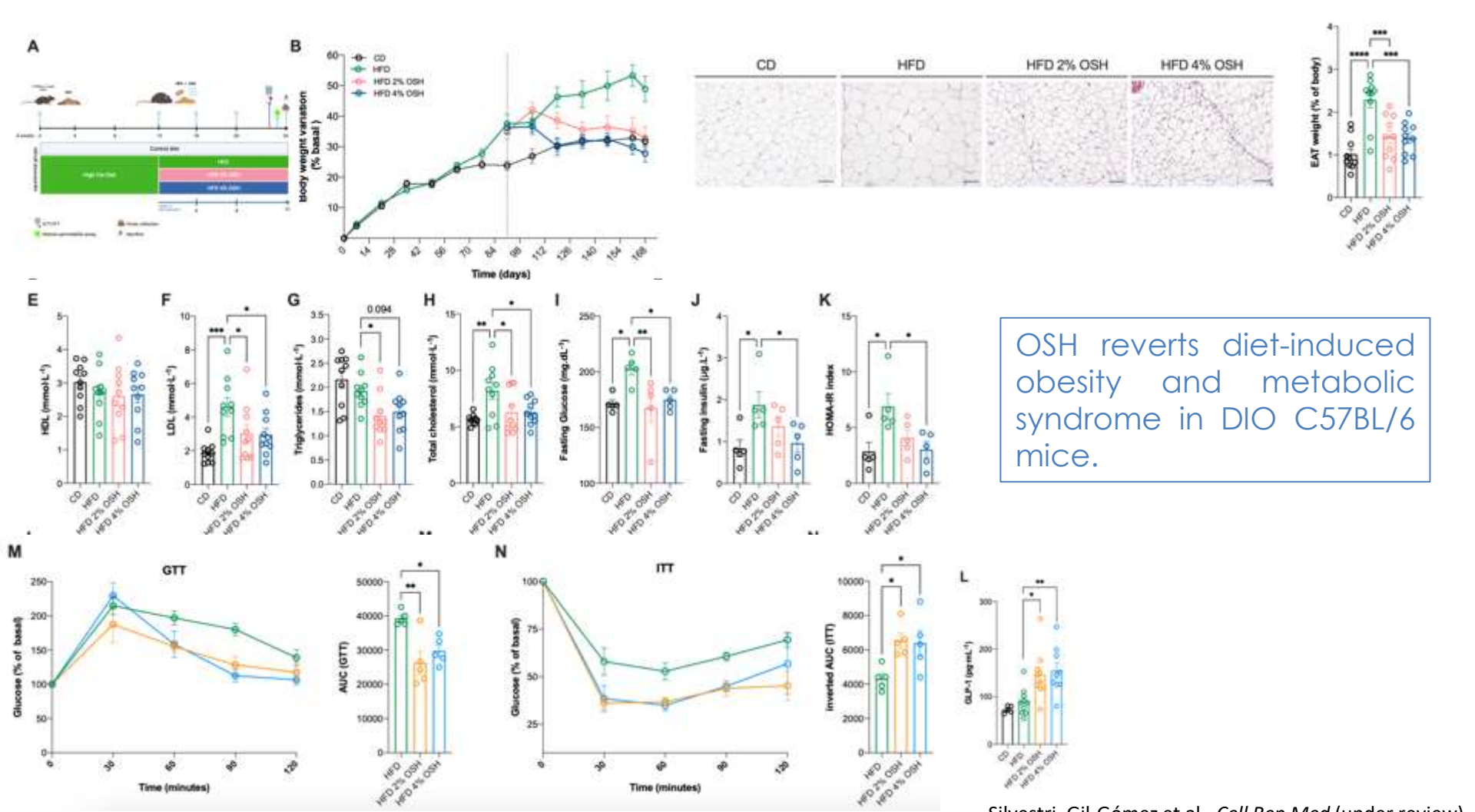
synthesized from
naturally-derived building blocks

MODIFIED CELLULOSE + CITRIC ACID



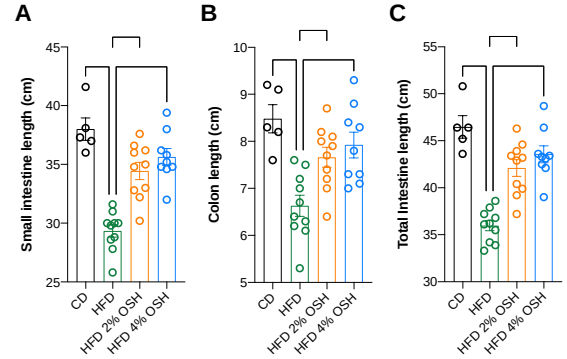


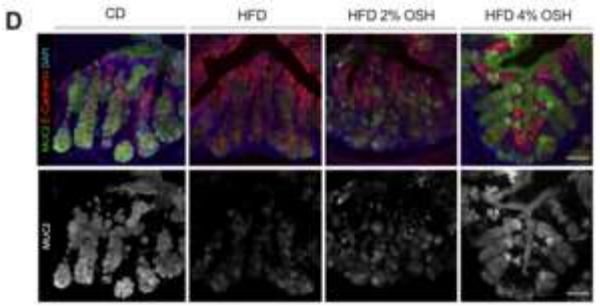
Madaghiele et al., *Sci Rep* 2021; Silvestri, Gil-Gómez et al., *Cell Rep Med* (under review)

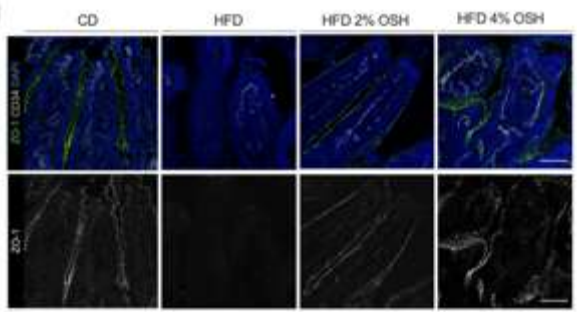


OSH reverts diet-induced obesity and metabolic syndrome in DIO C57BL/6 mice.

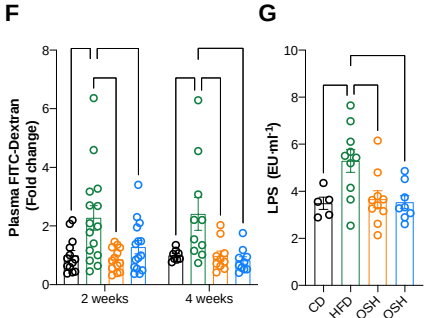
Silvestri, Gil-Gómez et al., *Cell Rep Med* (under review)

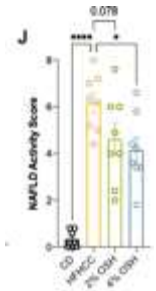


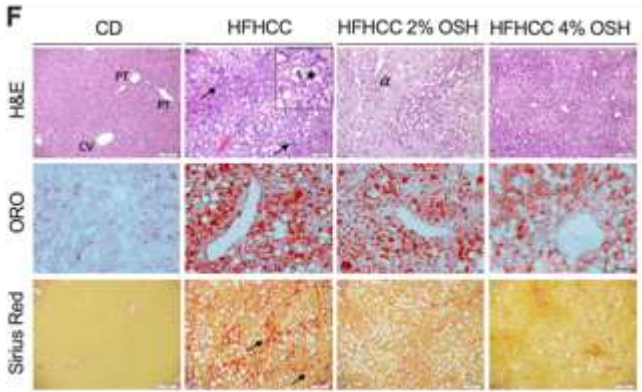


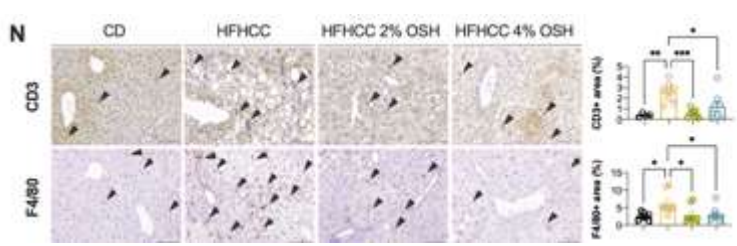


OSH preserves gut barrier integrity protecting from HFD-induced "leaky-gut".



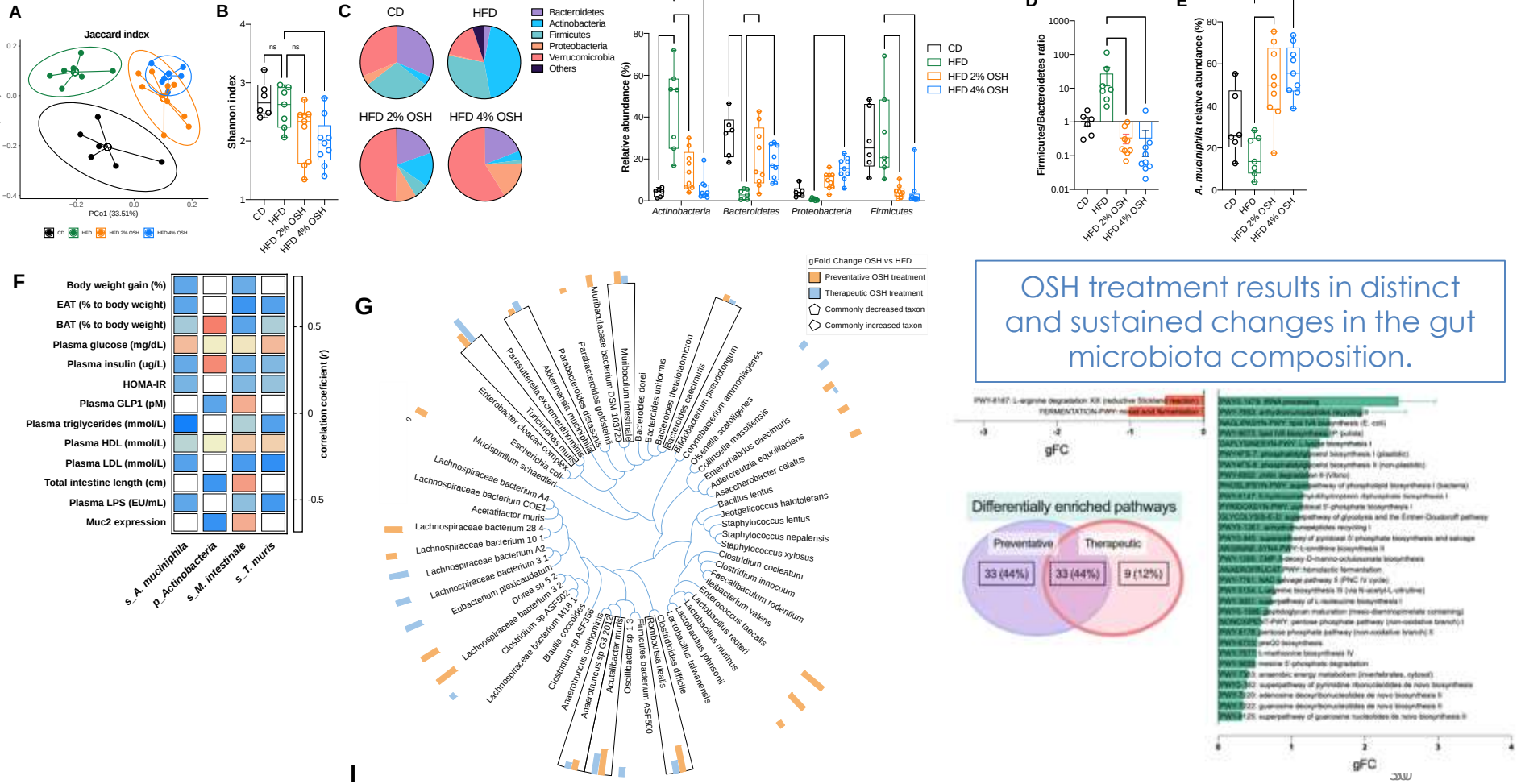




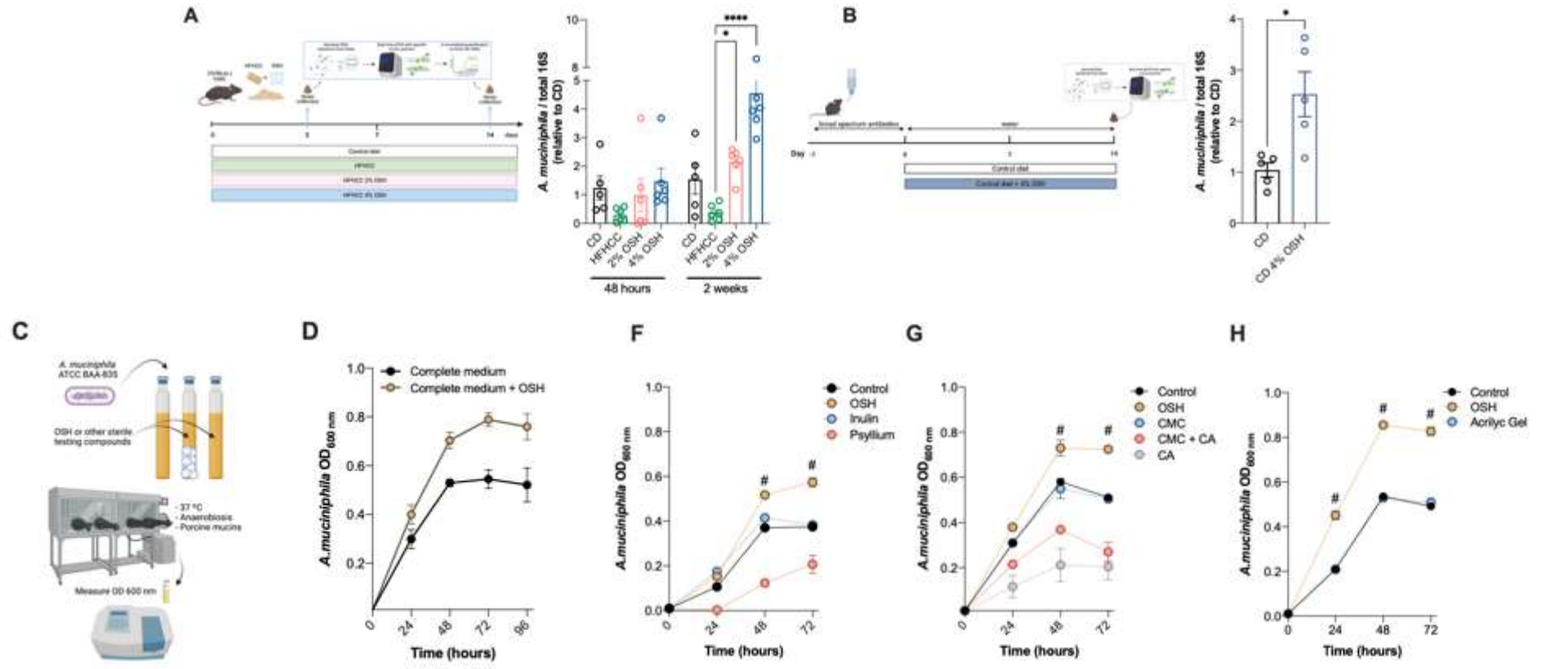


OSH limits the progression of non-alcoholic fatty liver disease.

Silvestri, Gil-Gómez et al., *Cell Rep Med* (under review)

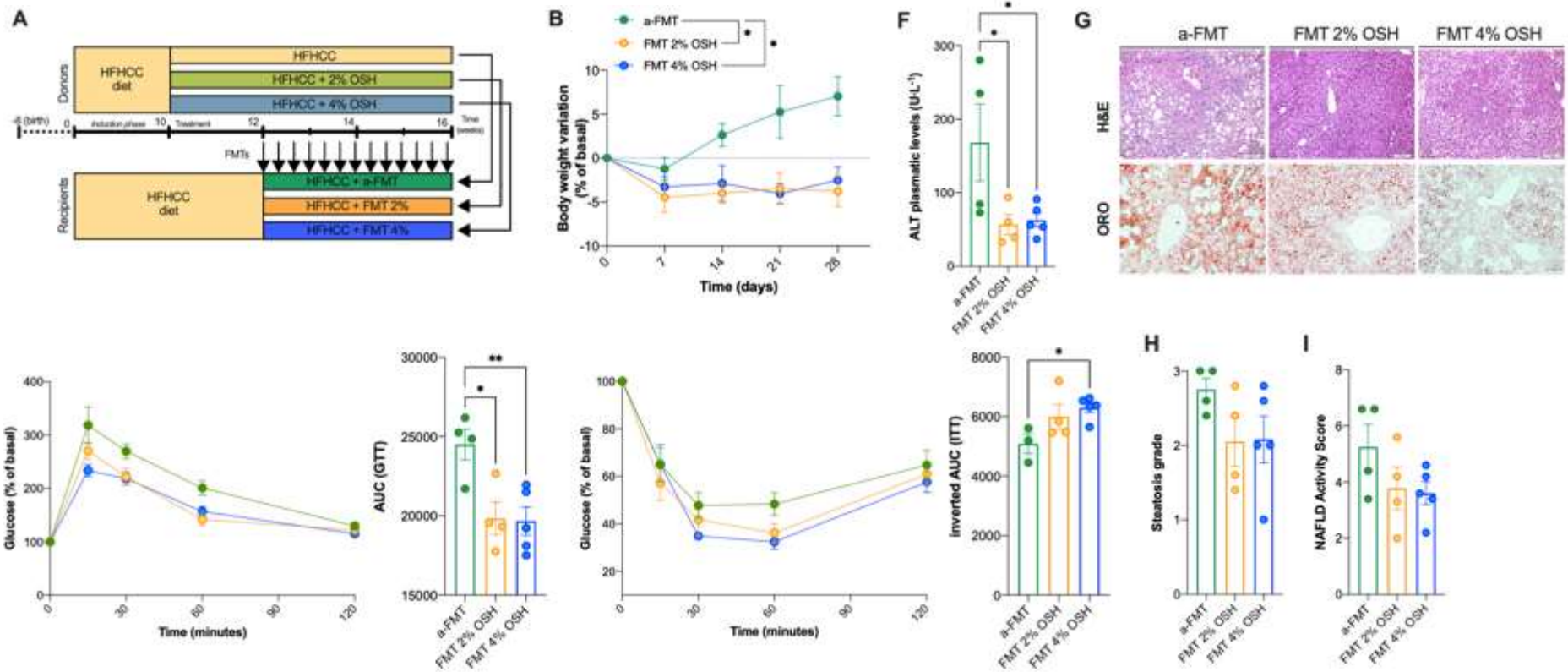


Silvestri, Gil-Gómez et al., *Cell Rep Med* (under review)



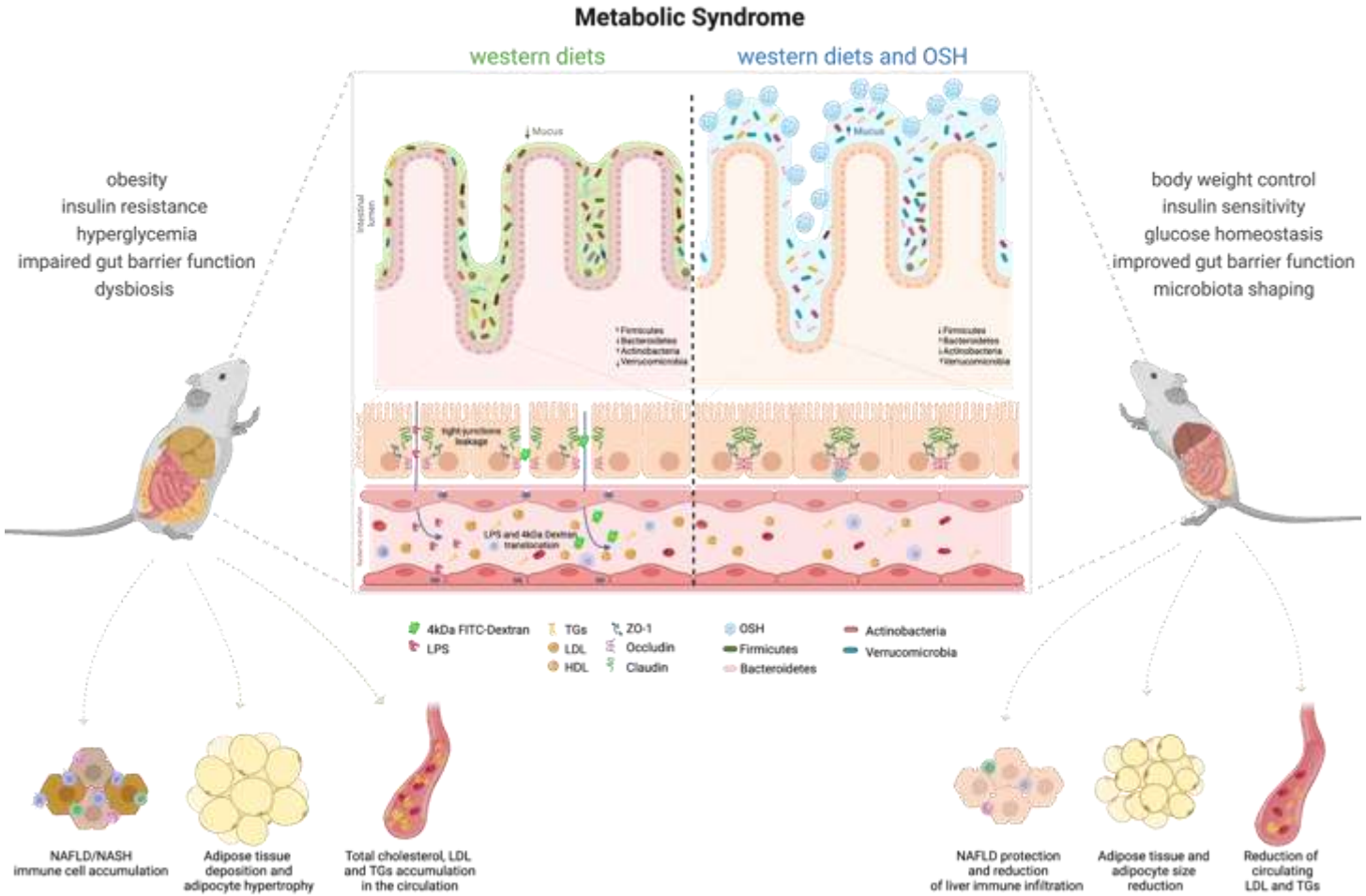
OSH fosters *Akkermansia muciniphila* growth

Silvestri, Gil-Gómez et al., *Cell Rep Med* (under review)



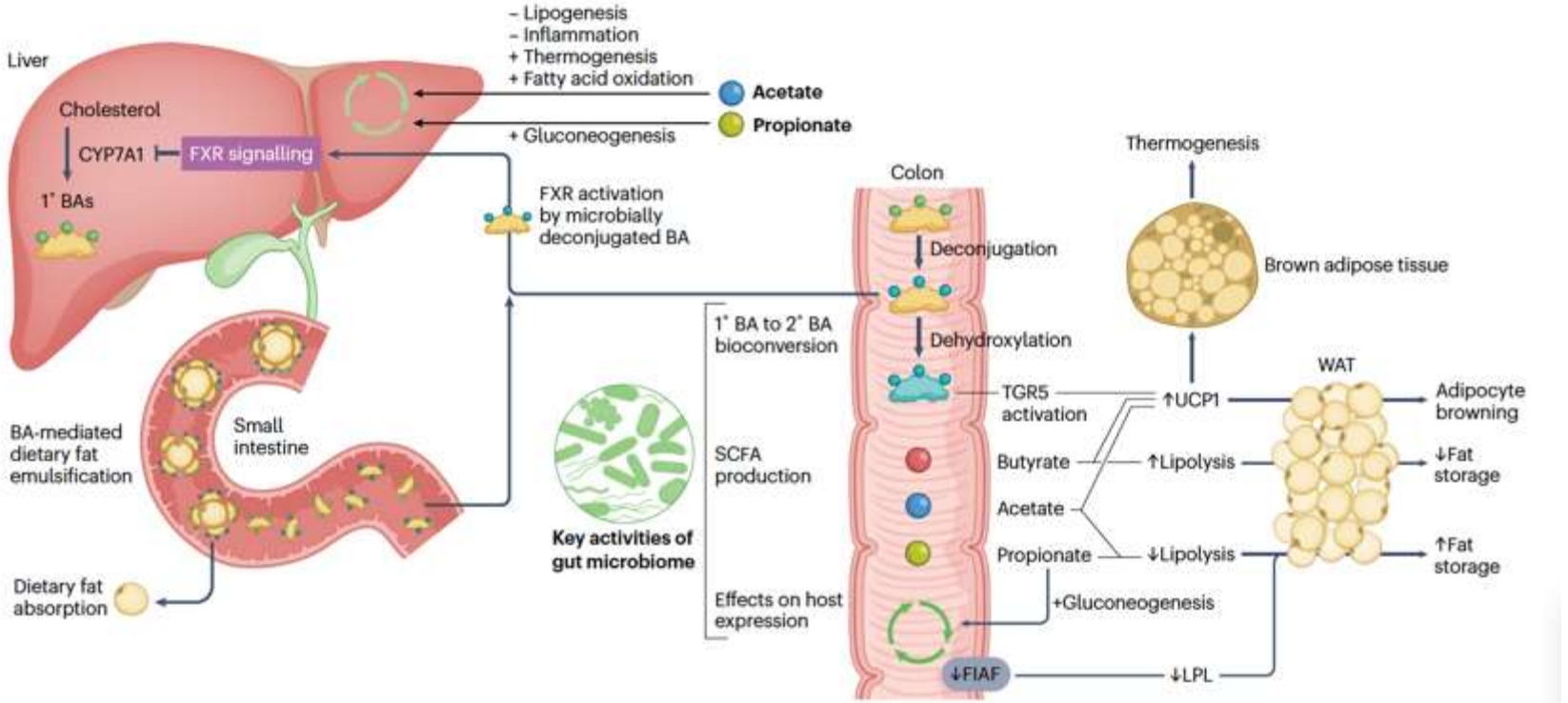
Gut microbiota drives metabolic benefits of OSH in mice. Recover upon fecal microbiota transplantation

Silvestri, Gil-Gómez et al., *Cell Rep Med* (under review)



Silvestri, Gil-Gómez et al., *Cell Rep Med* (under review)

CONCLUSIONES



Carmody RN et al. Nat Rev Microbiol 2023

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