



ORGANIZA:



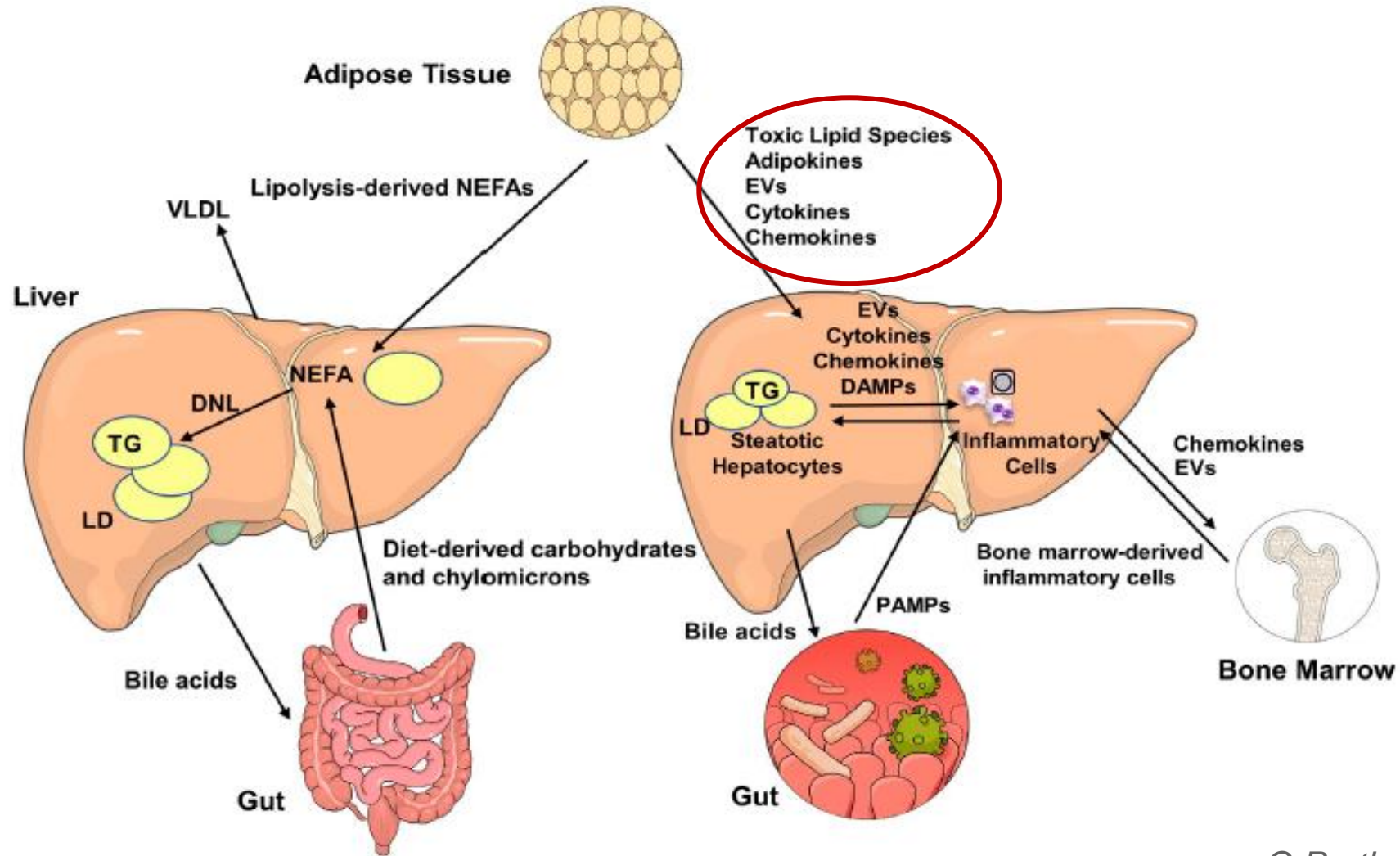
Asignatura 2: Hepatopatías metabólicas

El eje hígado-intestino en la enfermedad metabólica hepática por grasa

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Universidad de Alcalá, CIBERehd, Madrid

Metabolic interorgan crosstalk in MAFLD

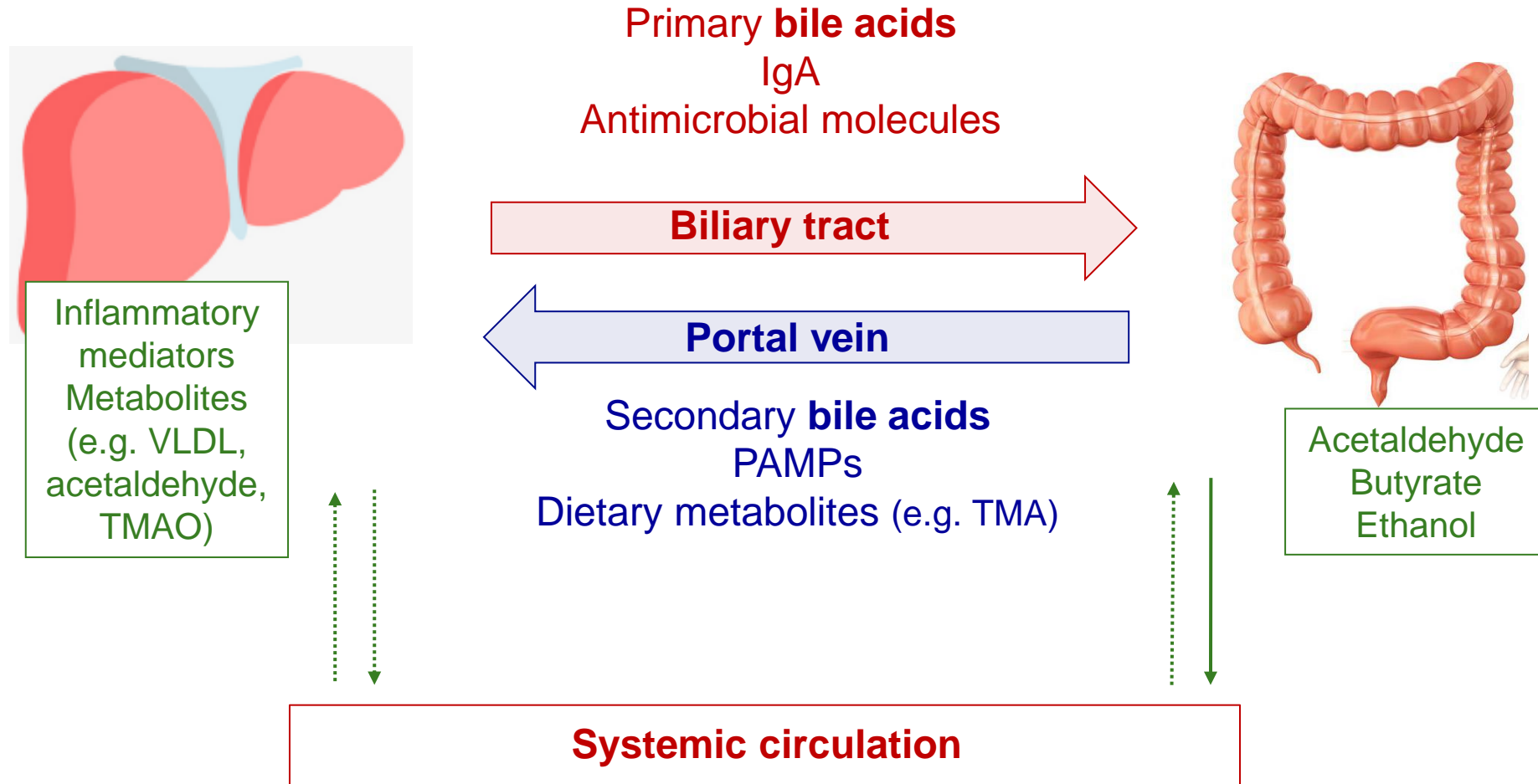


“Eje hígado-intestino en la enfermedad metabólica hepática por grasa”

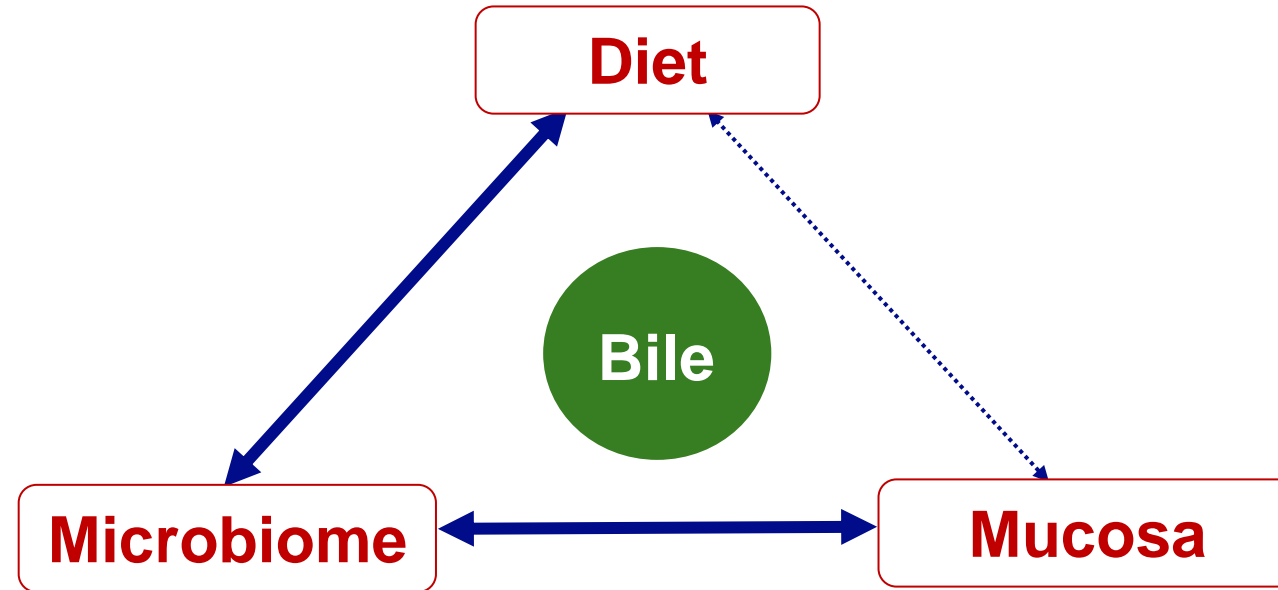
*G Parthasarathy et al.
Hepatol Com 2019*

The gut-liver axis:

A bidirectional crosstalk of information between the liver and gut



The gut-liver axis: A host (mucosa)-diet-microbiome interplay





- Intestinal microbiome
- Intestinal barrier
- Bacterial products and metabolites
- Gut-centric therapies

Causal role of the gut microbiome in hepatic steatosis

Preclinical studies in murine models



– Mice lacking gut microbiota are resistant to diet induced hepatic steatosis

Bowel decontamination improves hepatic steatosis in mice (AM Rutenburg et al, JEM 1957)

Germ-free mice are protected to hepatic steatosis and obesity (F Backhed et al, PNAS 2004)

– Transmissibility of hepatic steatosis via fecal microbiota transplantation

More steatosis in germ-free mice transplanted with feces from an obese than a lean donor (PJ Tumbau et al, Nature 2006)

Microbial transfer exacerbates NASH in mice (J Henao-Mejía et al, Nature 2012)

– Contribution of products/metabolites of gut microbiota to hepatic steatosis

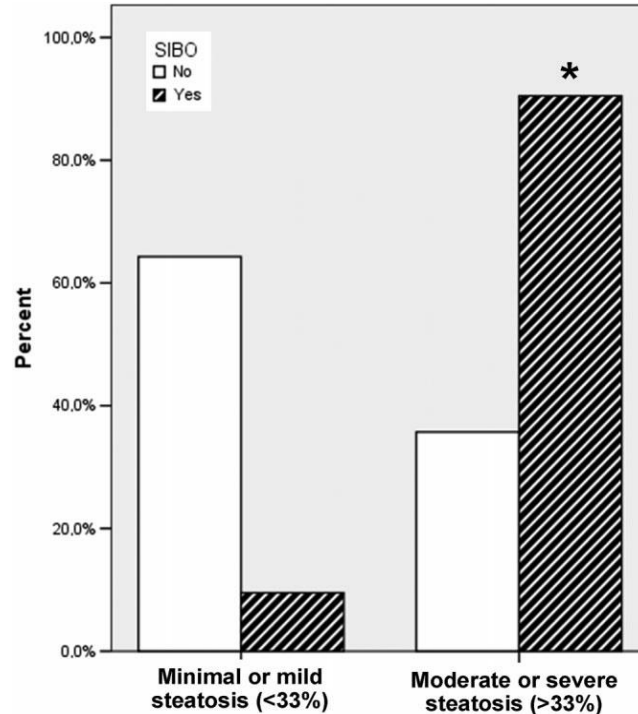
Increased energy harvest by the microbiota of obese (J Henao-Mejía et al, Nature 2012)

TLR4 is required for steatosis in mice (M Saberi et al, Cell Metabol 2009)



Increased small intestinal bacterial overgrowth (SIBO) in patients with MAFLD

SIBO in NAFLD (Glucose breath test)



SIBO in MAFLD (Culture of duodenal aspirates)

32 patients with MAFLD

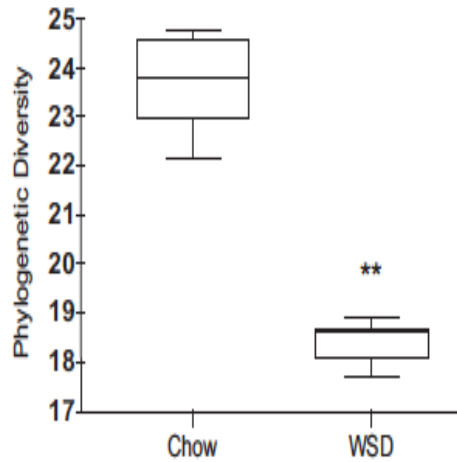
- SIBO in 12/32 (**38%**)
- NASH >F2: SIBO 16% vs. no SIBO 15%

Monomicrobial SIBO in 92%, mostly E. coli

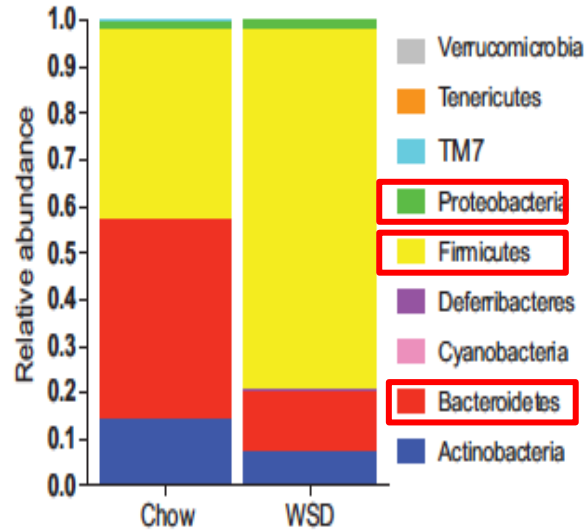
- Correlation of SIBO, permeability and MetS (→ LPS)
- No correlation with NASH

Changes in colonic lumen and mucosa microbiota in mice on a western style diet (WSD)(~HFD, 8wks)

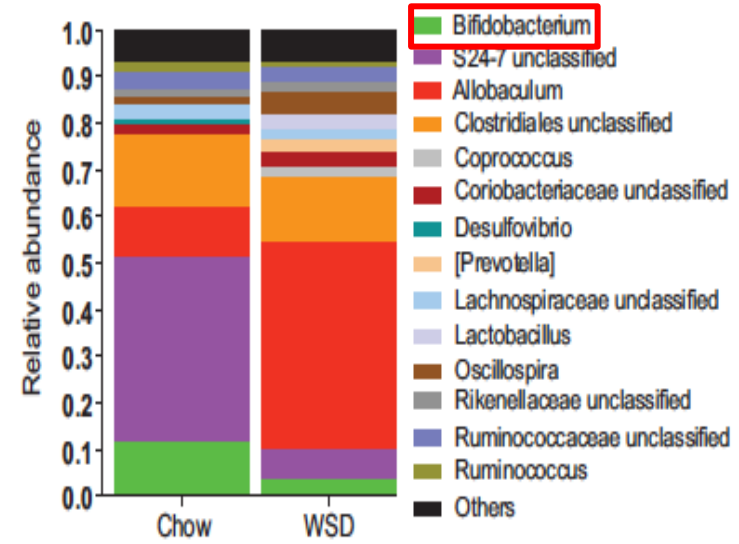
Phylogenetic diversity



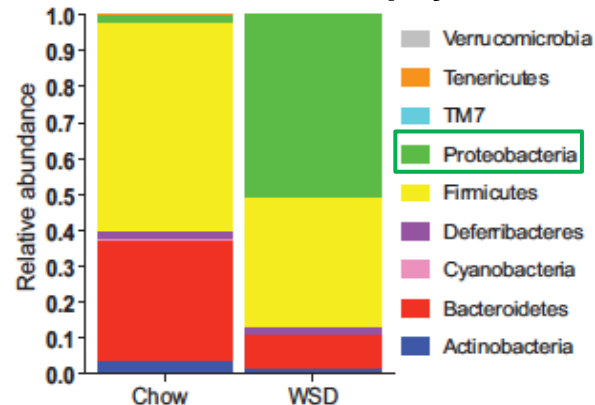
Colonic lumen - phylum



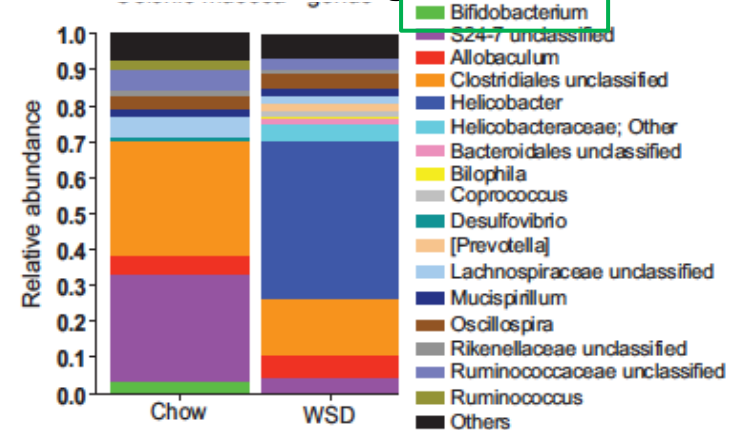
Colonic lumen - genus



Colonic mucosa - phylum



Colonic mucosa - genus



Diagnostic test accuracy of fecal-microbiome-derived metagenomic signature to detect advanced fibrosis in NAFLD in a clinical setting

Gut microbiome composition by **whole-genome shotgun DNA sequencing** from **stool** samples

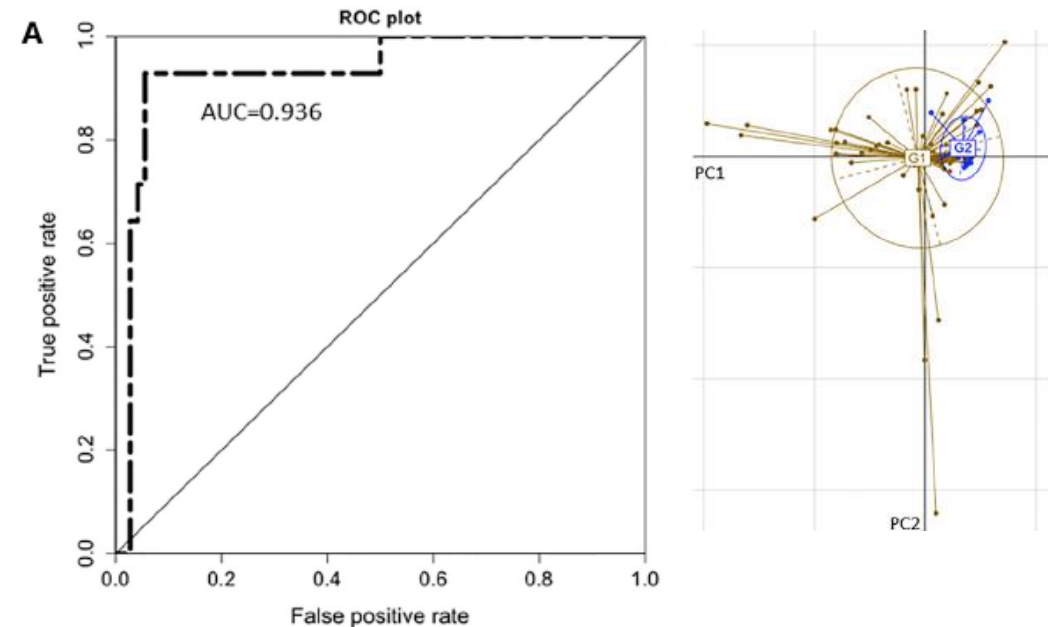
86 patients with biopsy-proven NAFLD:

- 72 mild/moderate (G1)
- 14 advanced fibrosis (G2)

Composition of microbiome

Phylum	Mild/moderate NAFLD	Advanced fibrosis	p Value
	G1 Median, SD	G2 Median, SD	
Firmicutes	58.81% (20.8)	42.61% (23.9)	0.01520
Proteobacteria	1.85% (15.3)	4.54% (22.9)	0.04004
Bacteroidetes	23.62% (18.1)	28.46% (27.4)	0.57840
Actinobacteria	2.67% (4.1)	2.02% (7.4)	0.78340
Species			
<i>Ruminococcus obeum</i> CAG:39	0.06% (0.54)	0.01% (0.02)	0.00005*
<i>Ruminococcus obeum</i>	0.29% (0.90)	0.11% (0.15)	0.00009*
<i>Eubacterium rectale</i>	2.56% (5.66)	0.12% (1.35)	0.00009*
<i>Faecalibacterium prausnitzii</i>	1.63% (4.07)	0.34% (3.07)	0.01961
<i>Escherichia coli</i>	0.29% (15.8)	0.99% (25.3)	0.44330
<i>Bacteroides vulgatus</i>	1.76% (4.56)	2.19% (7.04)	0.85610

Performance of the model to differentiate mild/moderate and advanced fibrosis

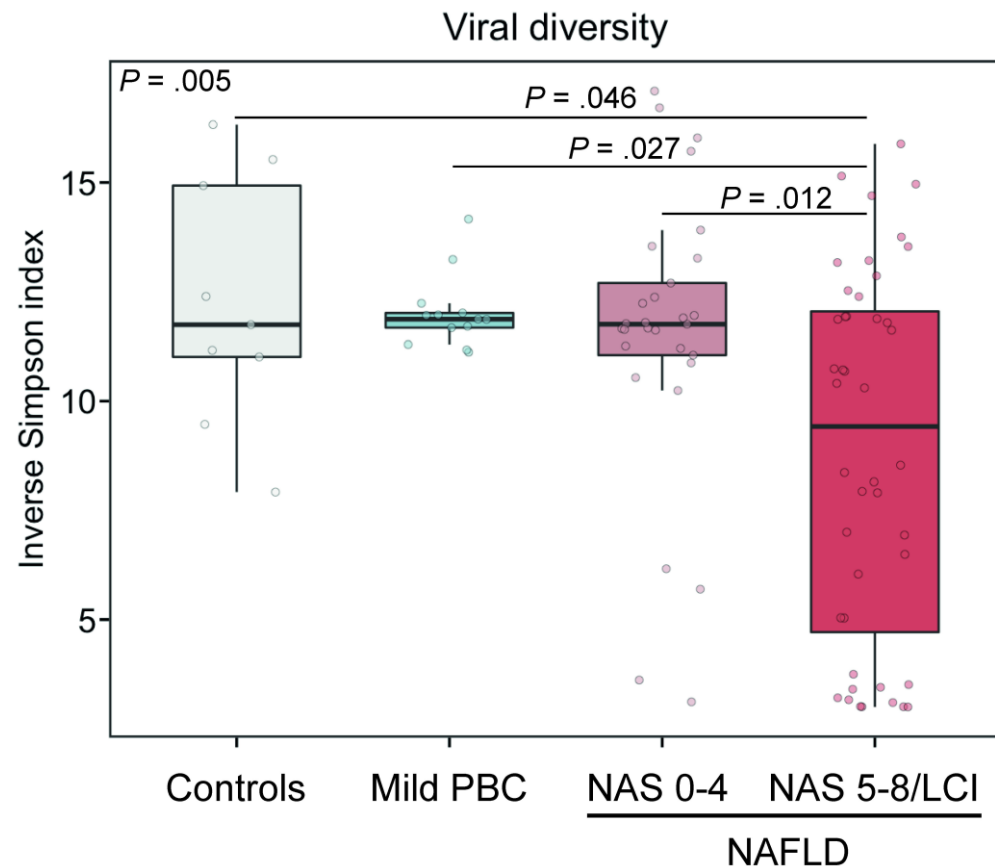


- NS differences in abundance of serum metabolites and gut microbial gene pathways between groups

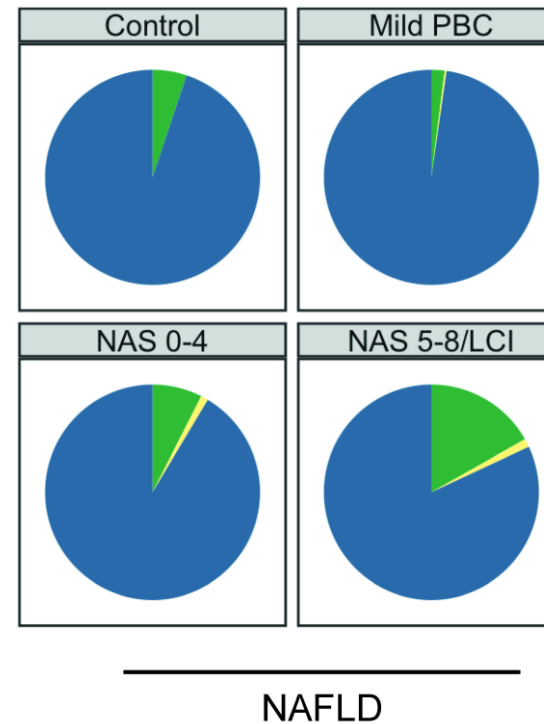
Intestinal virome signature is associated with NAFLD severity

73 patients with biopsy-proven NAFLD:

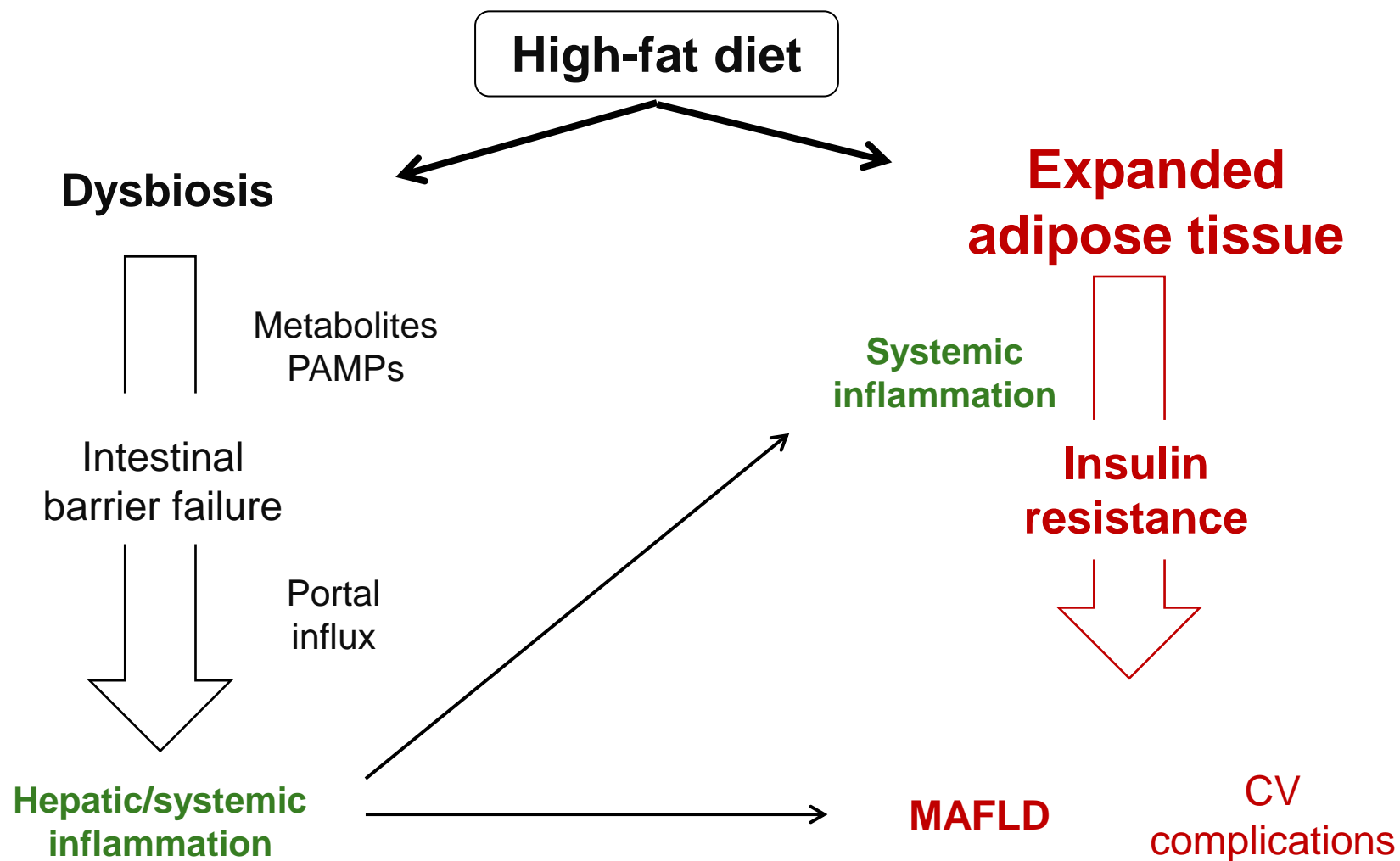
- 37 mild/moderate (F0-F1)
- 36 advanced fibrosis (F2-F4)



■ Bacteriophages
■ Mammalian viruses
■ Other viruses, including plant/food derived viruses



Intestinal barrier failure as a permissive factor of portal influx of bacterial products/metabolites from gut dysbiosis

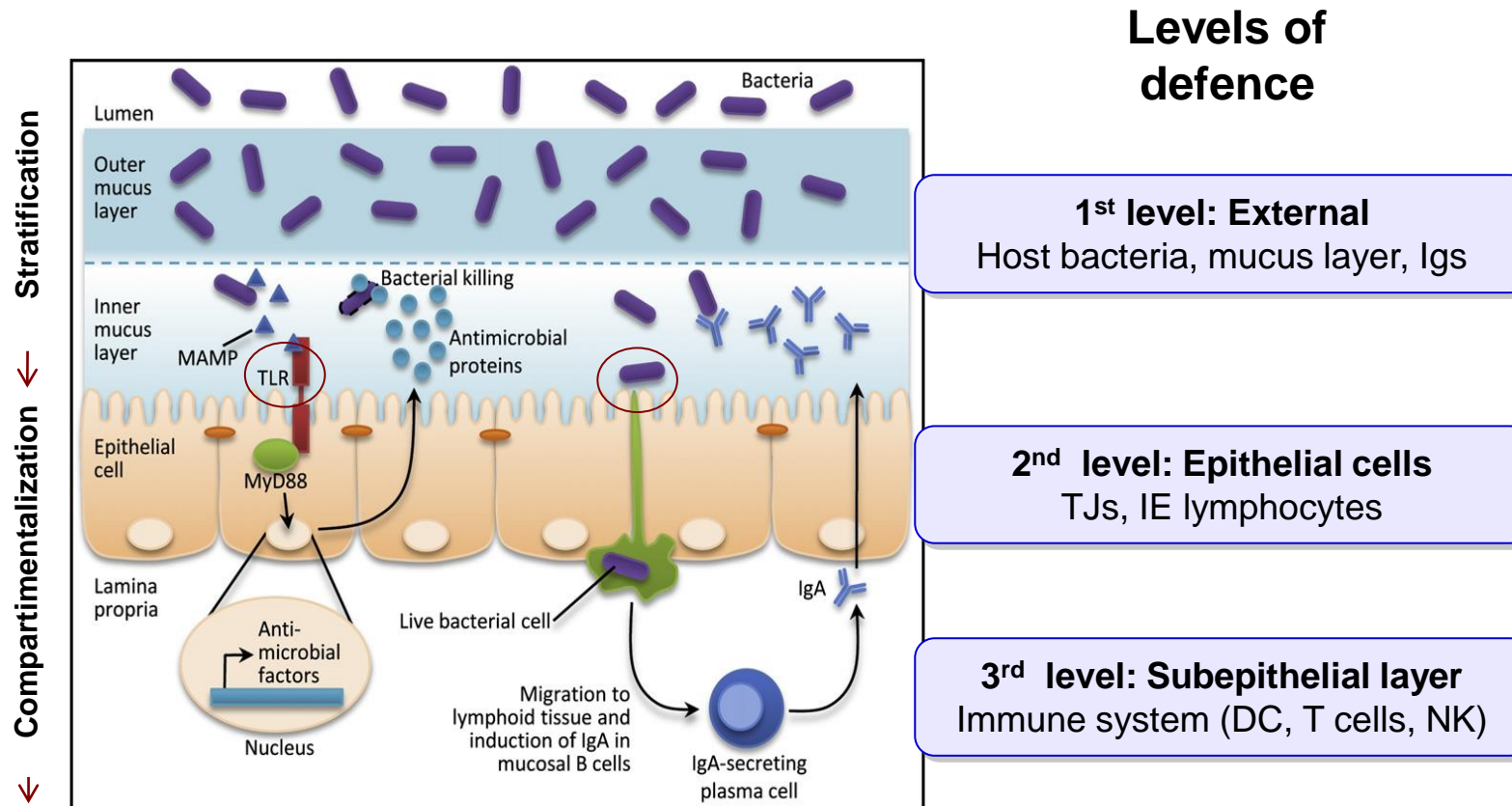


“Eje hígado-intestino en la enfermedad metabólica hepática por grasa”



- Intestinal microbiome
- **Intestinal barrier**
- Bacterial products and metabolites
- Gut-centric therapies

Structure of the intestinal barrier

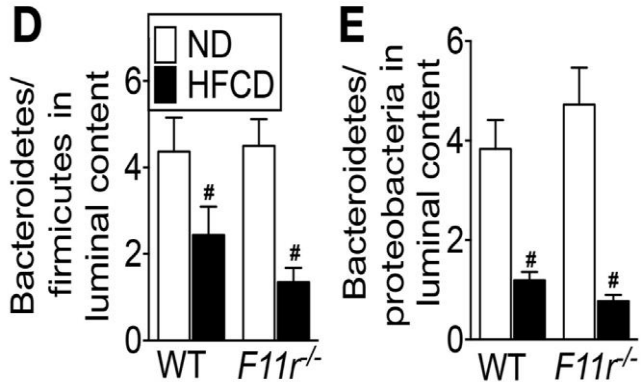


Modified from BA Duerkop et al. Immunity 2009

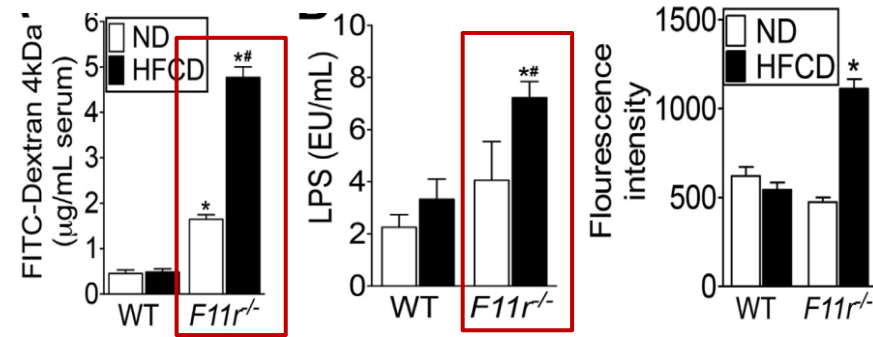
Loss of junctional adhesion molecule A (Jam1) promotes severe steatohepatitis and increased endotoxemia in mice on a HFCD

Amelioration of liver inflammation with antibiotics

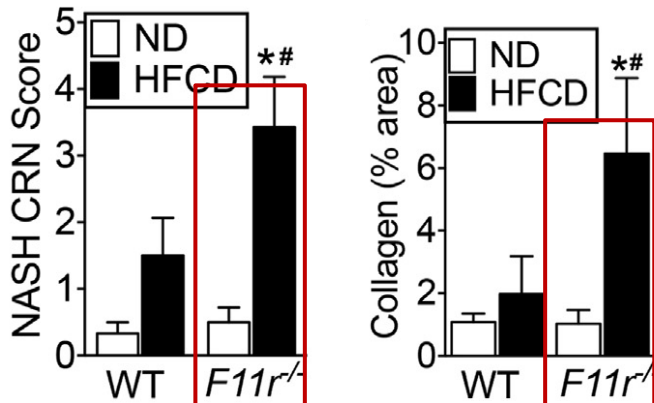
Microbiome disturbance



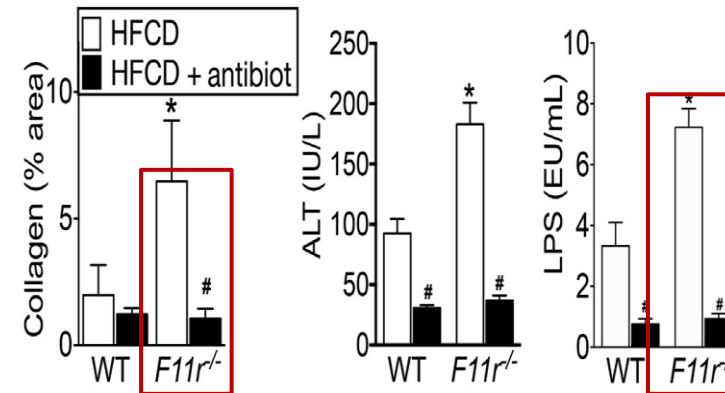
Intestinal permeability, endotoxemia and inflammation



Hepatic inflammation and fibrosis



Effect of antibiotics on hepatic collagen and ALT and LPS

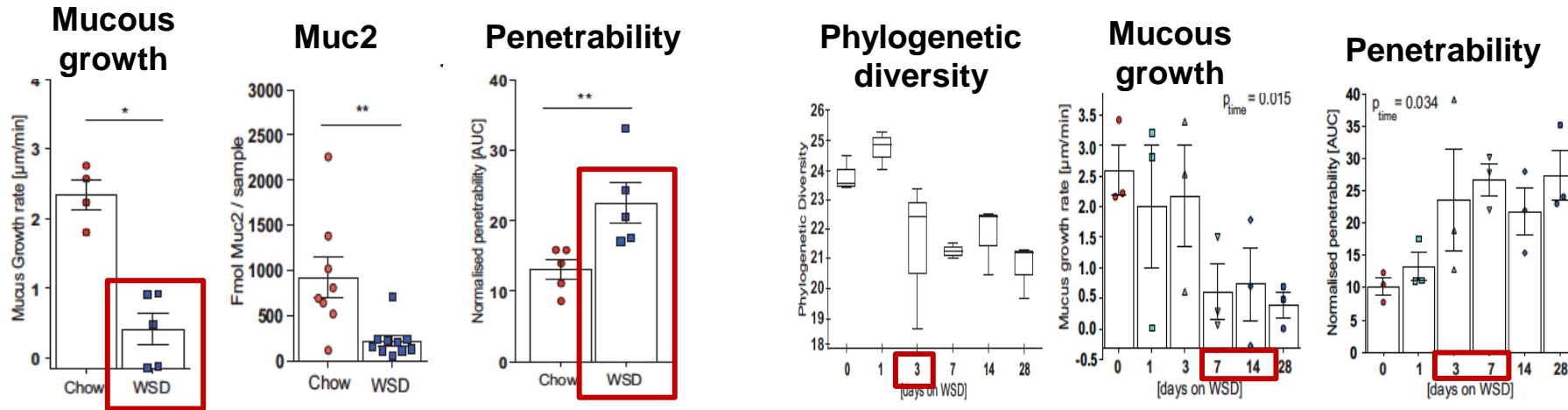


“Eje hígado-intestino en la enfermedad metabólica hepática por grasa”

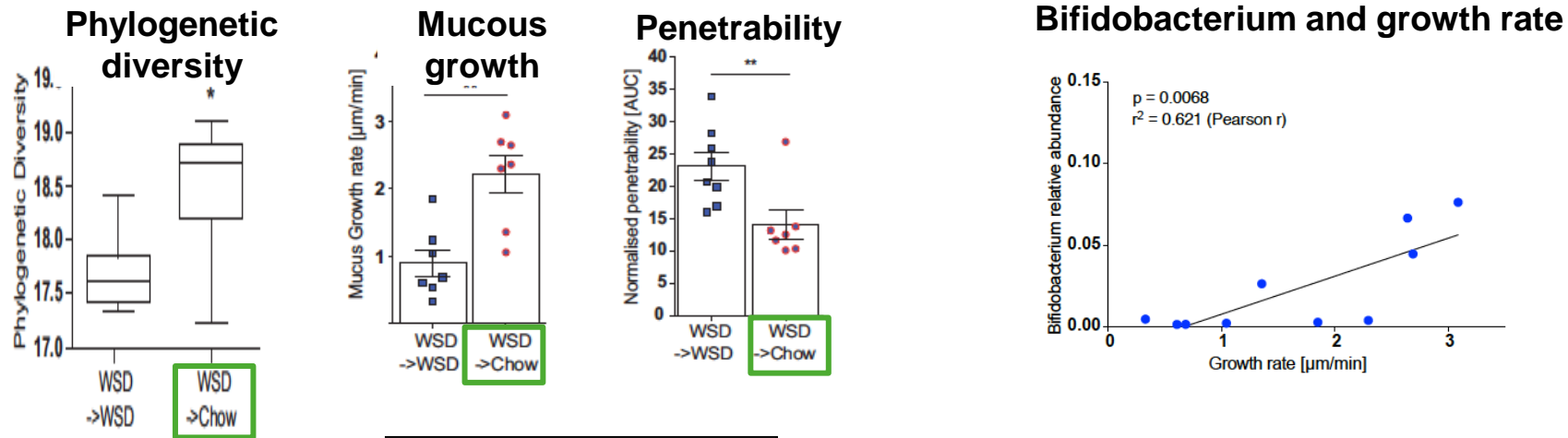
K Rahman et al. Gastroenterology 2016

Defects in colonic mucous layer of mice on a low-fiber western-style diet (WSD)(8 wks)

Amelioration by fecal microbiota transplantation



Fecal microbiota transplantation from chow-fed mice



Cecum SCFA:

- Reduced under WSD
- Unchanged after FMT

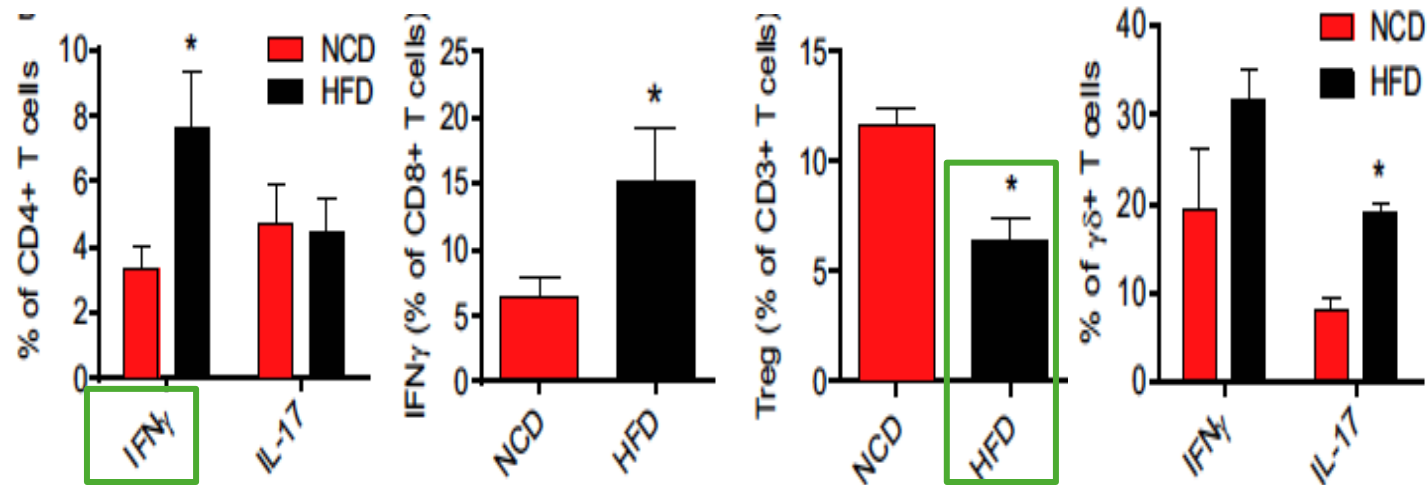
“Eje

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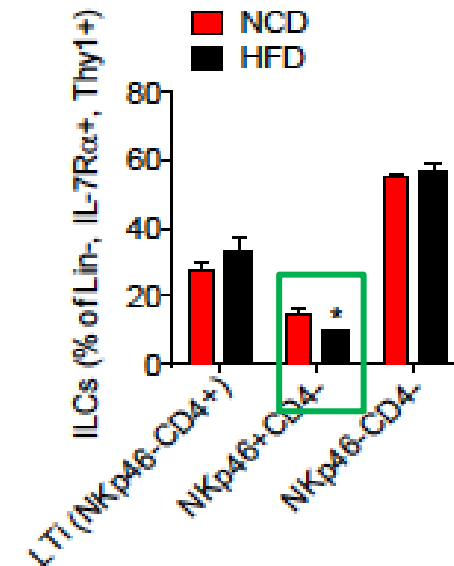
BO Schroeder et al. Cell Host Microb 2018

High-fat diet (HFD) induces low-grade (subclinical) inflammatory changes in intestinal resident immune cells

Pro-inflammatory shift of intestinal immune cells

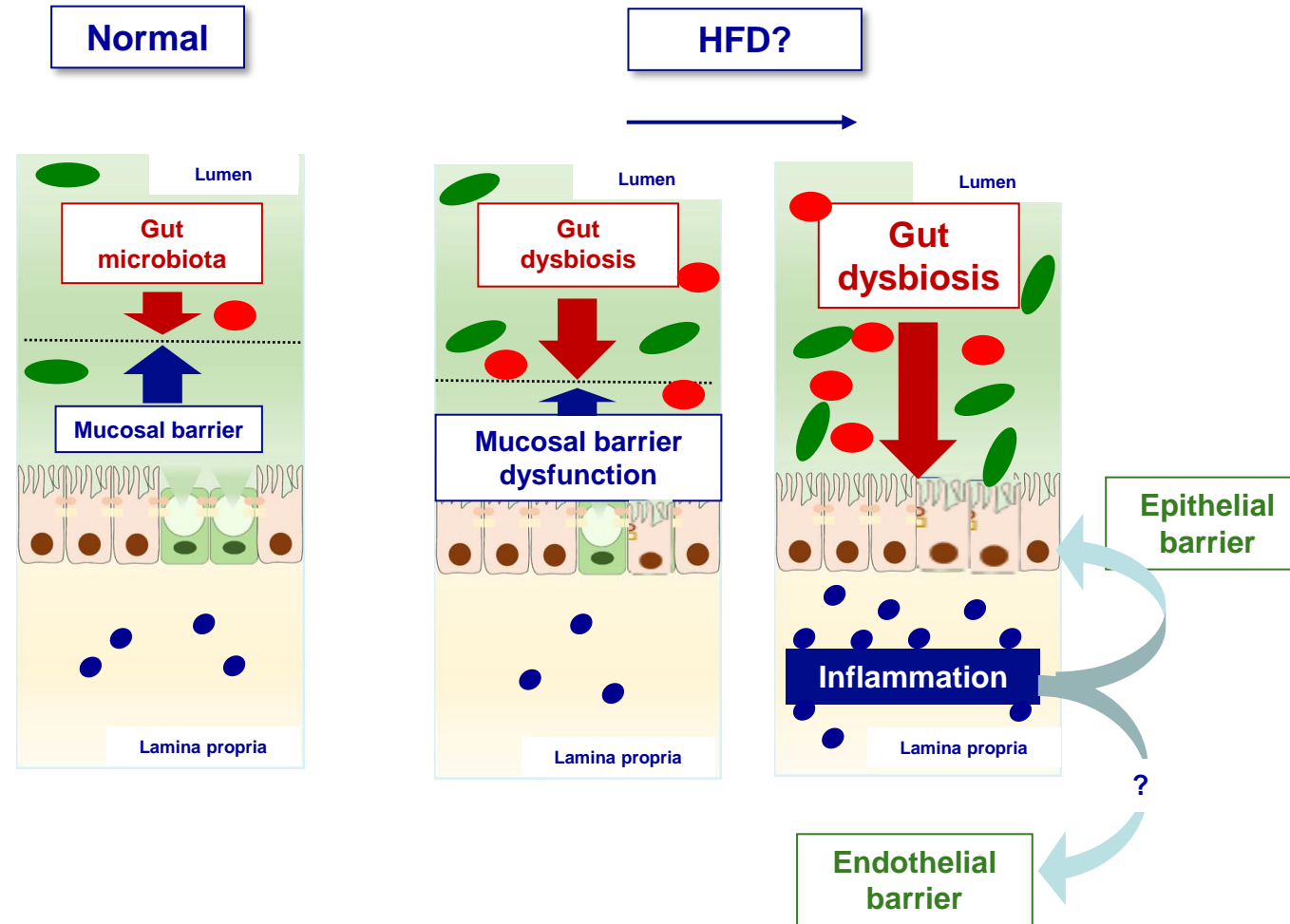


Reduced IL-22 producing ILC (innate lymphoid cells)



H Luck et al. Cell Metabolism 2015

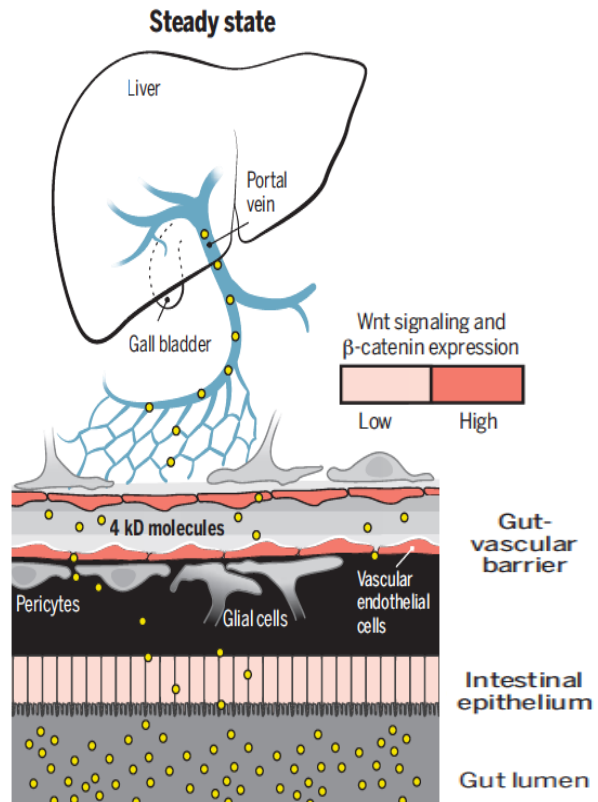
An imbalance between mucosal barriers and gut microbes promotes intestinal inflammation in NAFLD



“Eje hígado-intestino en la enfermedad metabólica hepática por grasa”

Selective dysruption of the gut-vascular barrier by HFD/MCDD

Correction by obeticholic acid

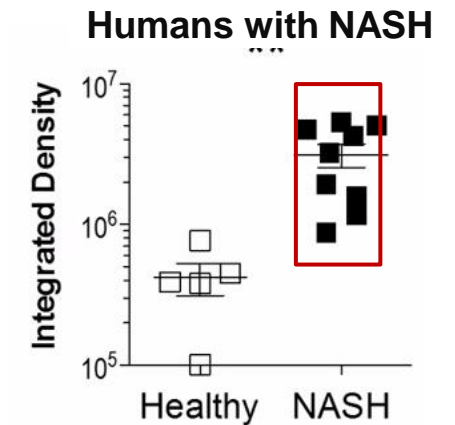
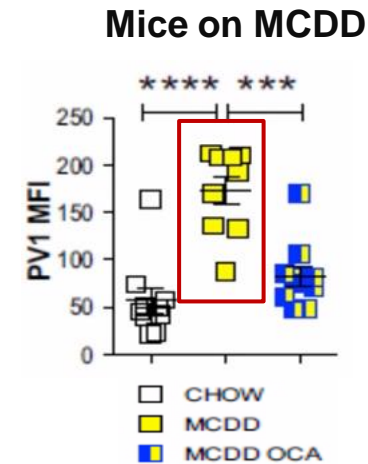
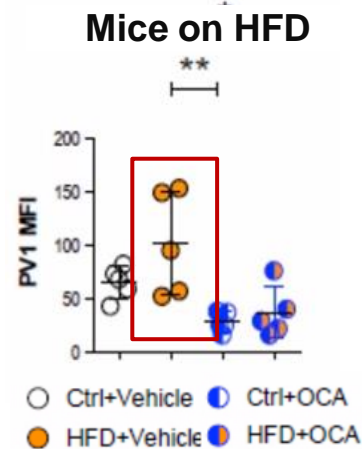


β -catenin inactivation

- \uparrow claudin-3
- \uparrow **PLVAP**
- BBB breakdown

PLVAP= plasmalemma vesicle associated protein

PV1 Expression in ileum



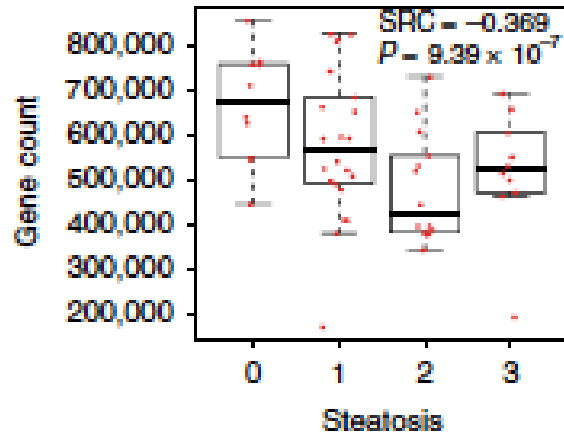


- Intestinal microbiome
- Intestinal barrier
- **Bacterial products and metabolites**
- Gut-centric therapies

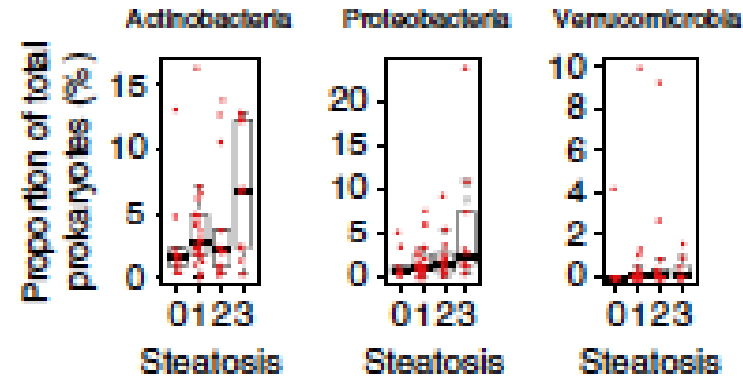
- **Bacterial components (PAMPs)**(LPS, mitochondrial DNA, flagellin)
 - Mice deficient in TLR-4/MD2 protected from MCD diet-induced hepatic steatosis/inflammation (T Csak et al, AJP 2011)
 - Mice deficient in TLR9 or on TLR9 antagonist protected from HFD-induced hepatic steatosis/inflammation (Garcia-Martinez I et al, JCI 2016)
- **Bacterial metabolites**
 - **SCFA** (butyrate, propionate, acetate) induced by high-fiber diet in the colon
 - contradictory results → differential abundance of individual SCFA
 - SCFA by high-fiber diet alleviate T2DM (L Zhao et al, Science 2019)
 - (Tri)methylamine (**TMA**) converted by microbiota from choline (ME Dumas et al, PNAS 2006)
 - **Ethanol** increase in blood in NASH children and NAFLD adults (1000 times lower) (L Zhu et al, Hepatology 2013. AJ Engstler et al, Gut 2016)
 - **Phenylacetic acid** (L Hoyles et al, Nature Medicine 2018)

Molecular networks between gut microbiome and hepatic steatosis phenotype in non-diabetic obese women from Spain and Italy

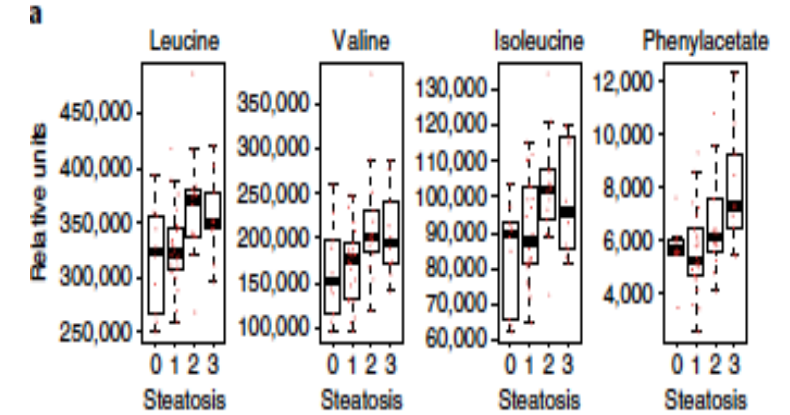
Microbial gene richness



Taxa correlated with liver steatosis



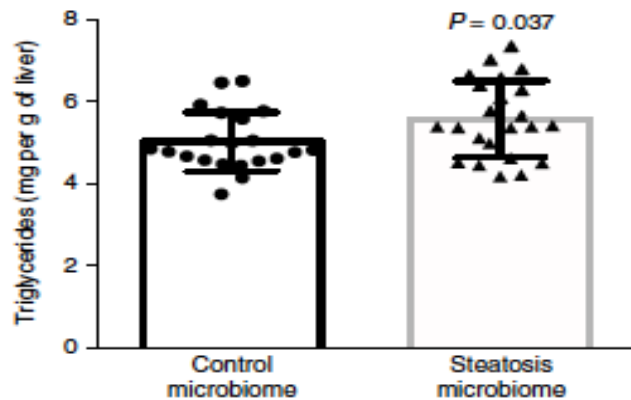
Plasma metabolites correlated with liver steatosis



- Similar metabolite pattern in urine
- Pathways involving AAA and BCAA

Transfer of steatotic and metabolic phenotypes to mice

Steatosis 3 patients → mice



Metagenome-derived KEGG pathway microbiome data:

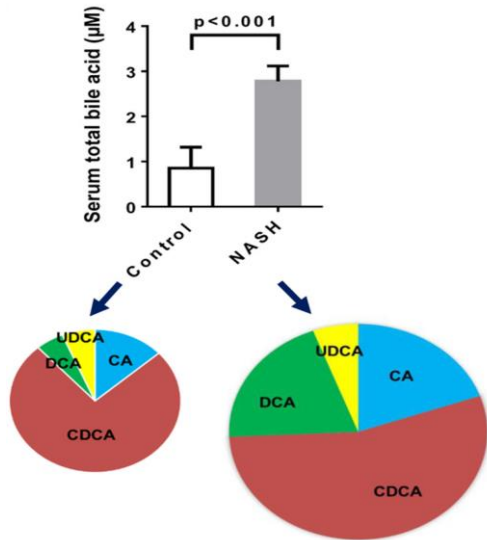
Correlations with liver steatosis ...

- LPS and peptidoglycan
- fatty acids
- BCA (valine, leucine, isoleucine)
- AAAs (tryptophan, tyr, phenylalanine)

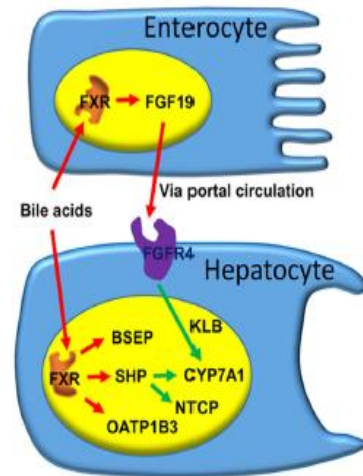
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Suppressed hepatic bile acid signaling despite elevated production of primary and secondary bile acids in MAFLD

Serum bile acids

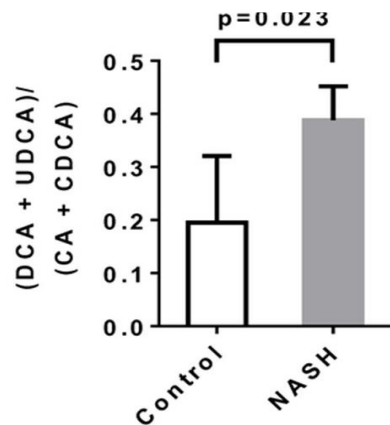


16 patients with NASH and 11 healthy controls

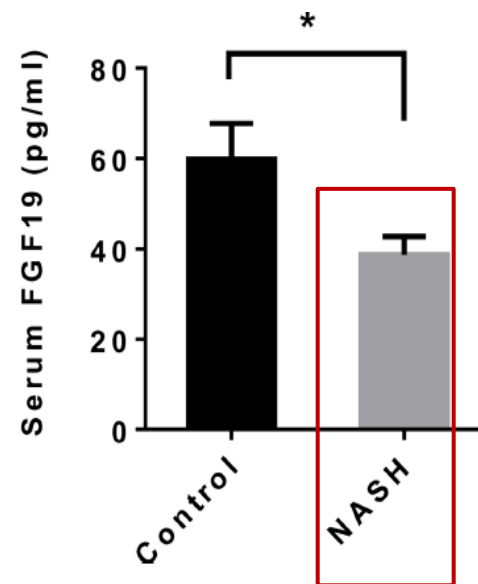


FXR affinity →
CDCA >>> DCA

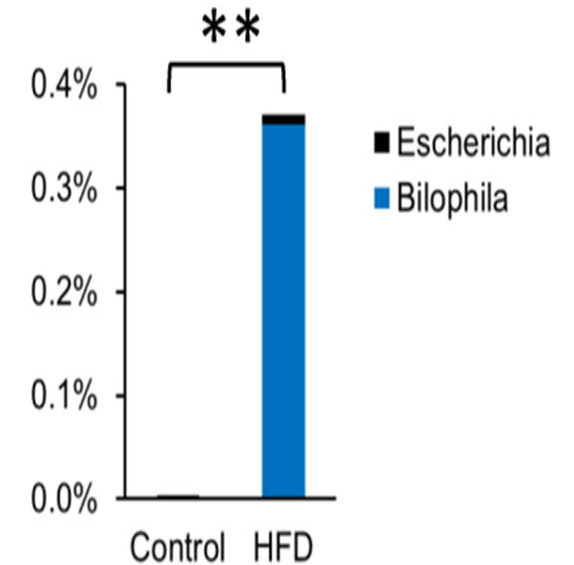
Secondary/primary BA ratio



Serum FGF19

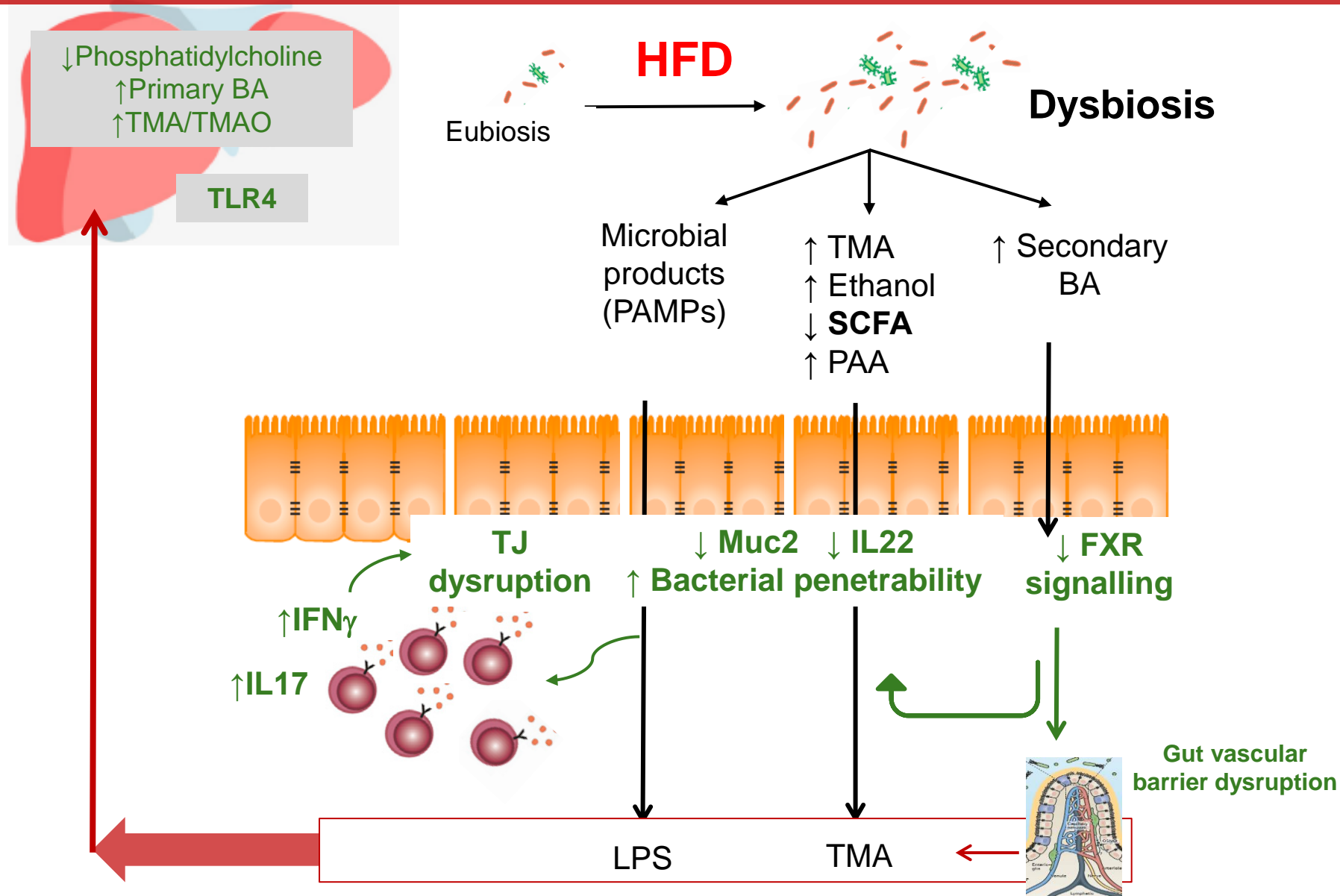


Microbiome composition Taurine metabolism



Gut-liver axis in MAFLD

Postulated mechanisms linking the gut microbiome, intestinal barrier dysfunction



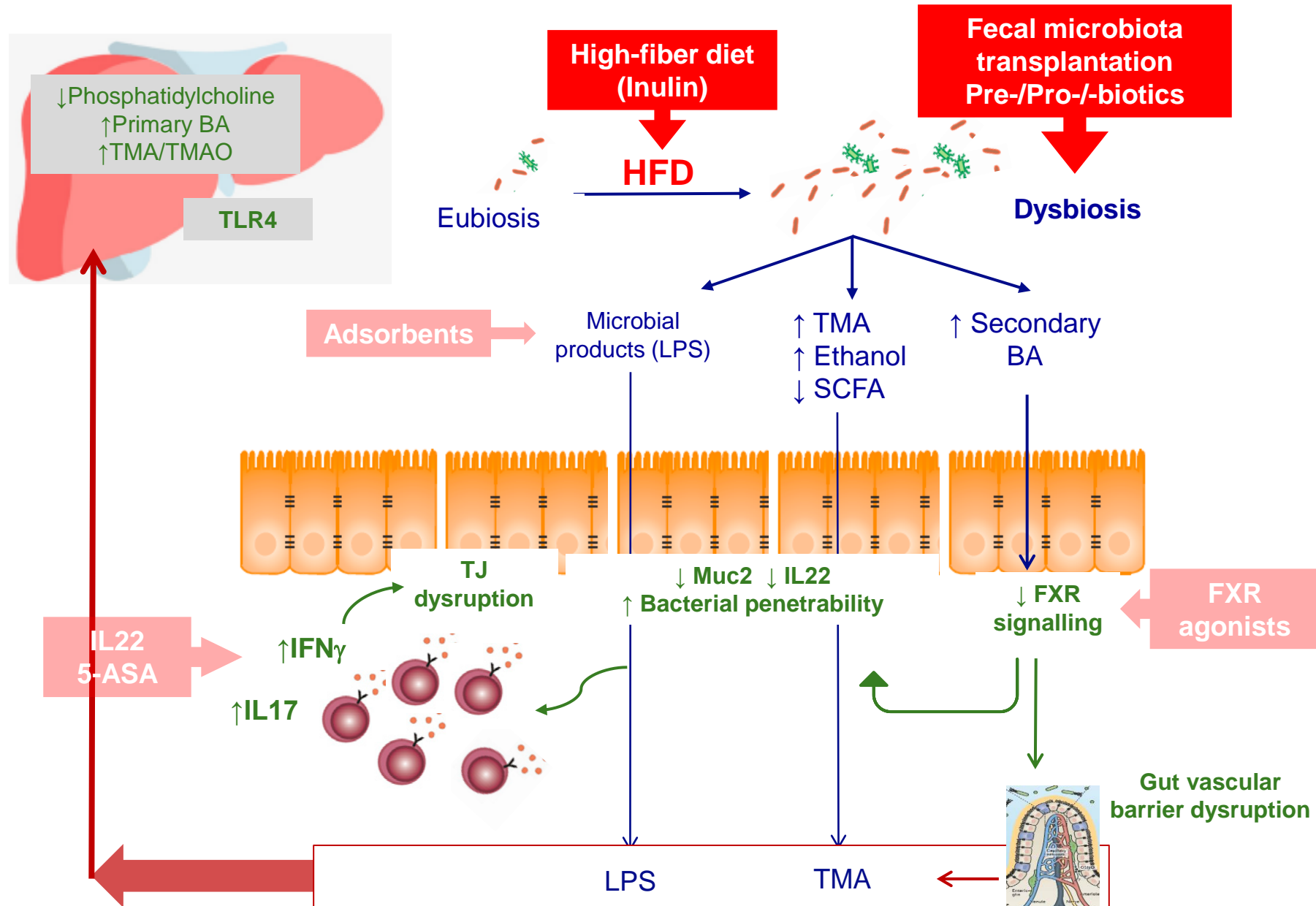
“Eje hígado-intestino en la enfermedad metabólica hepática por grasa”

A Albillos et al. JHEP 2019



- Intestinal microbiome
- Intestinal barrier
- Bacterial products and metabolites
- **Gut-centric therapies**

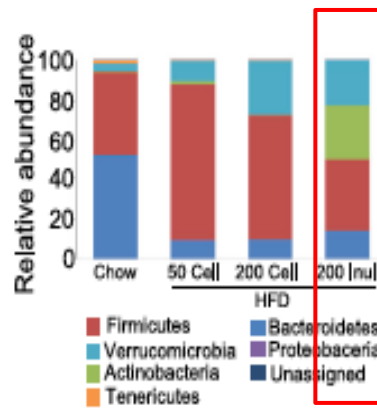
Gut-liver axis in MAFLD: Gut-centric therapies



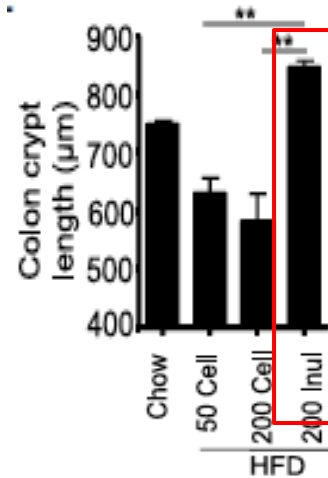
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Fermentable fiber (inulin) restores HFD-induced bacterial encroachment and colonic atrophy by a microbiota-dependent induction of IL-22

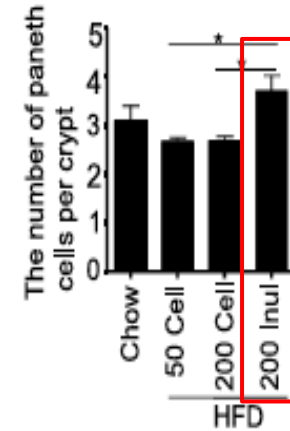
Fecal microbiota composition



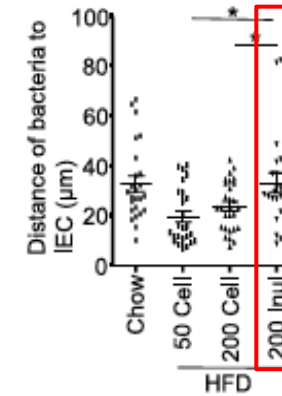
Colon atrophy



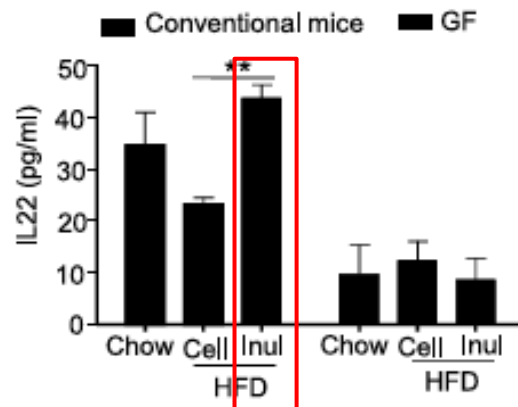
Paneth atrophy



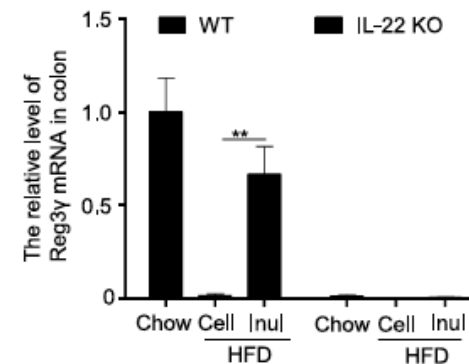
Reduced bacterial epithelial distance



Inulin induces IL-22 production by ILC3, not in GF mice



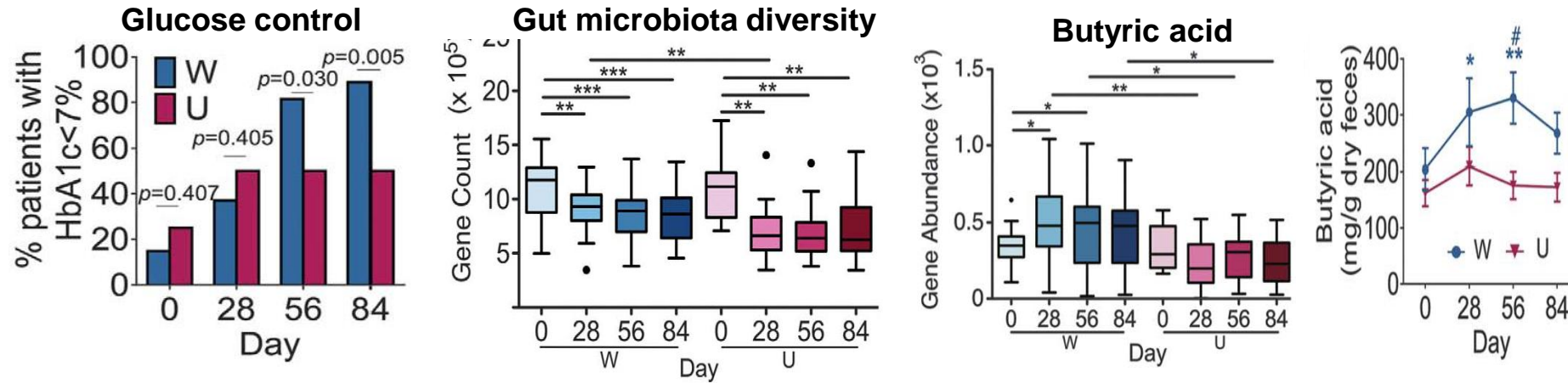
IL-22 mediated inulin increase in Reg3γ



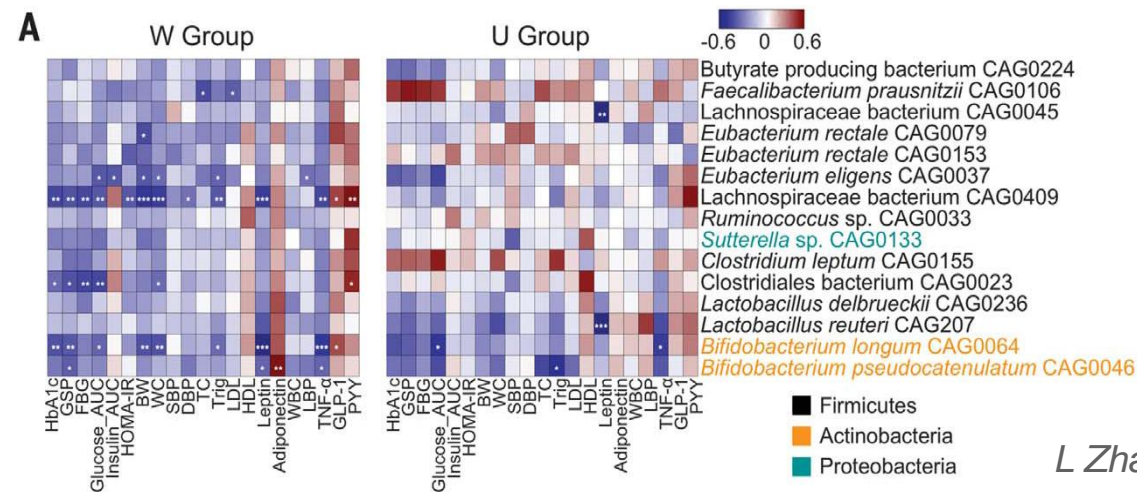
SCFA produced by carbohydrate fermentation of dietary fibers by gut bacteria alleviate T2DM

T2DM adults

Whole grains, high-fiber diet (**W**, 27 pts) vs. Usual diet (**U**, 16 pts)

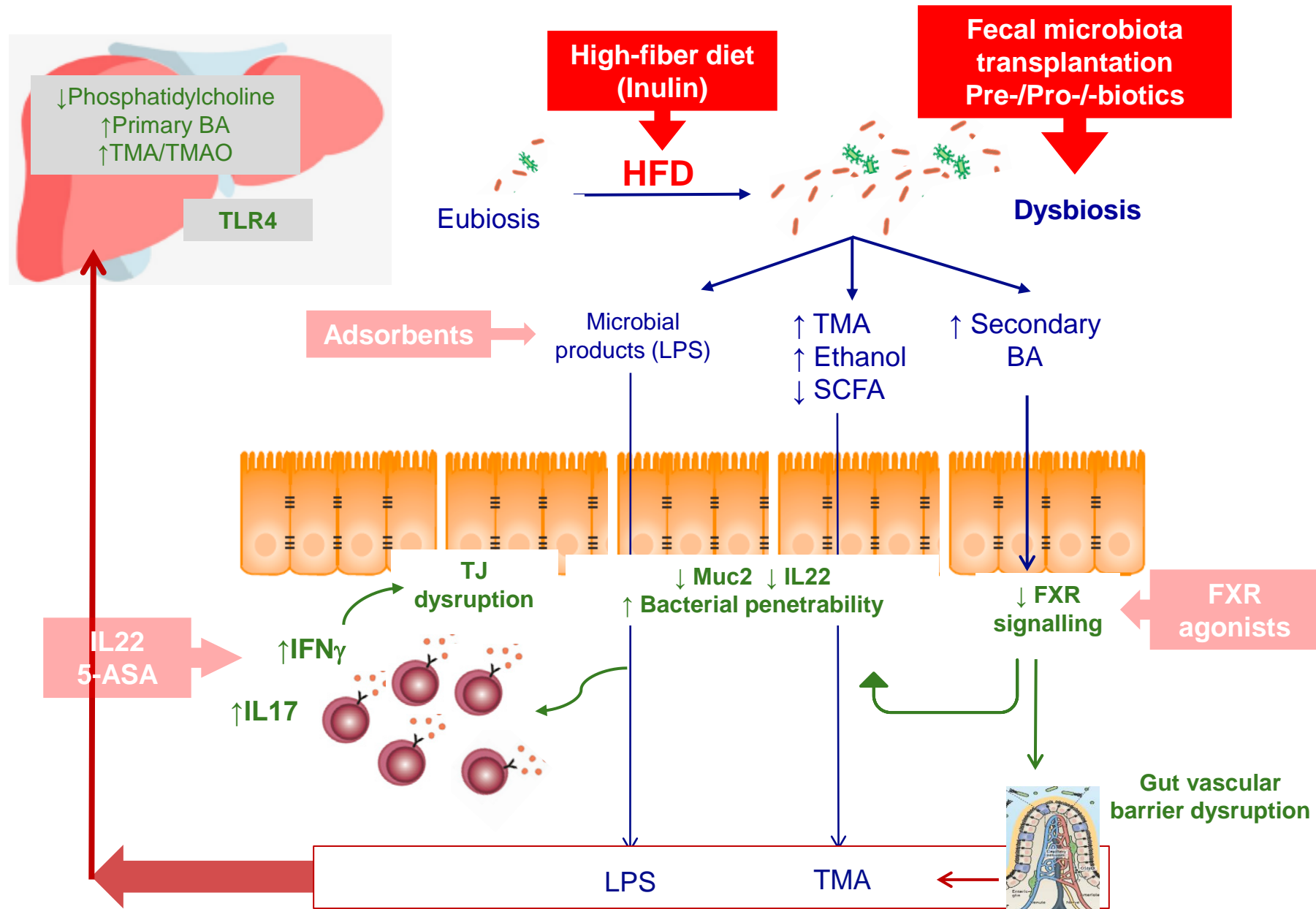


Heat map: correlations between the abundance of individual active SCFA producers (15 strains) and clinical metabolic pathways



L Zhao et al. Science 2019

Gut-liver axis in MAFLD: Gut-centric therapies



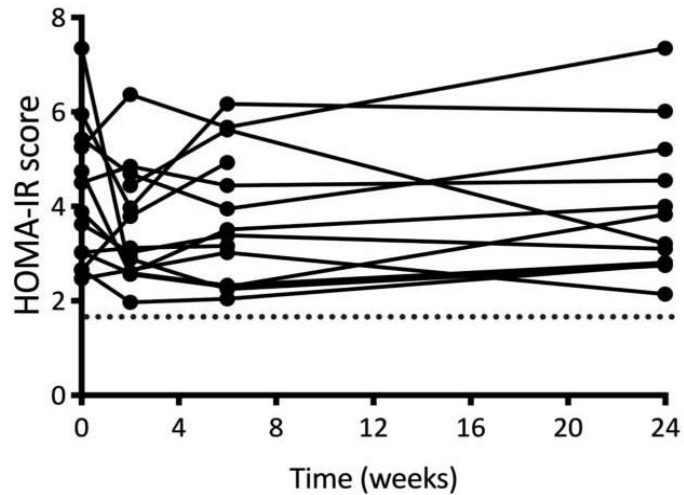
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FMT in MAFLD patients improves intestinal permeability, but not insulin resistance or hepatic steatosis

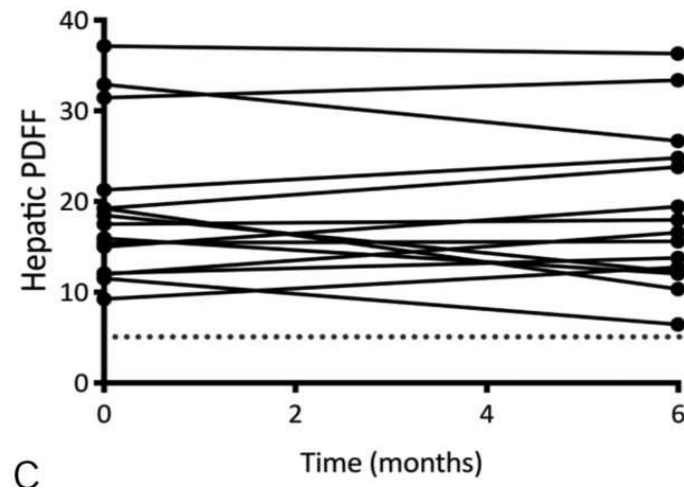
21 patients with NAFLD:

- 15 allogenic FMT from a thin and healthy donor
- 6 autologous

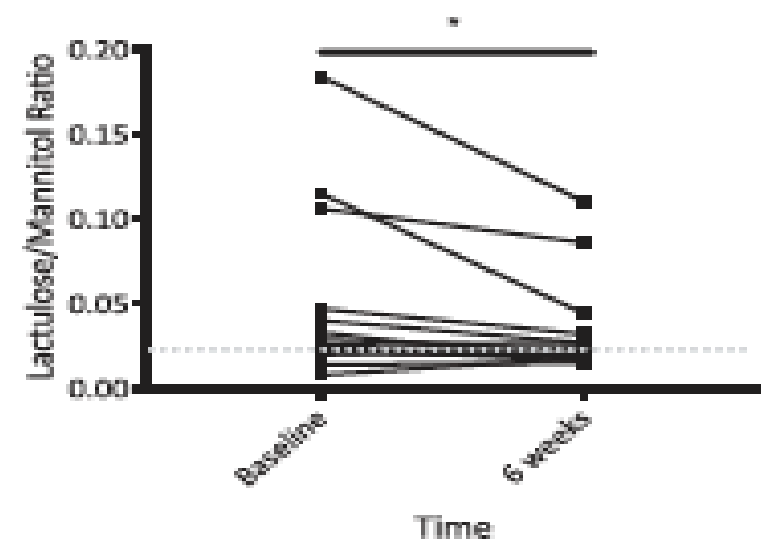
Insulin sensitivity



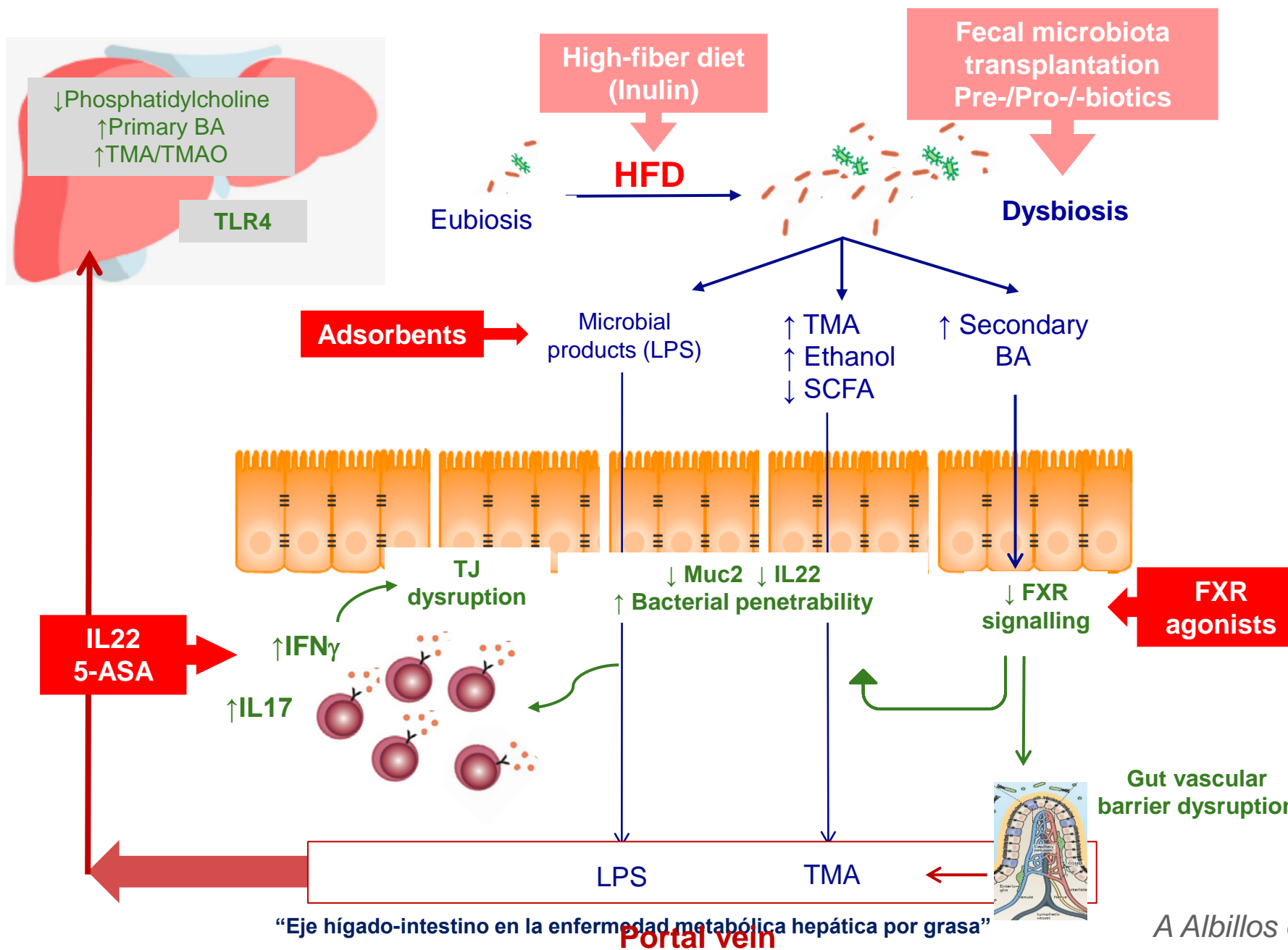
Hepatic steatosis



Intestinal permeability



Gut-liver axis in MAFLD: Gut-centric therapies



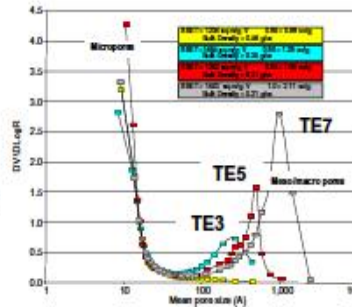
“Eje hígado-intestino en la enfermedad metabólica hepática por grasa”

Portal vein

A Albillos et al. JHEP 2019

Yaq-001, a synthetic activated carbon with tailor porosity, to adsorb gut-derived toxins and bacterial derived products

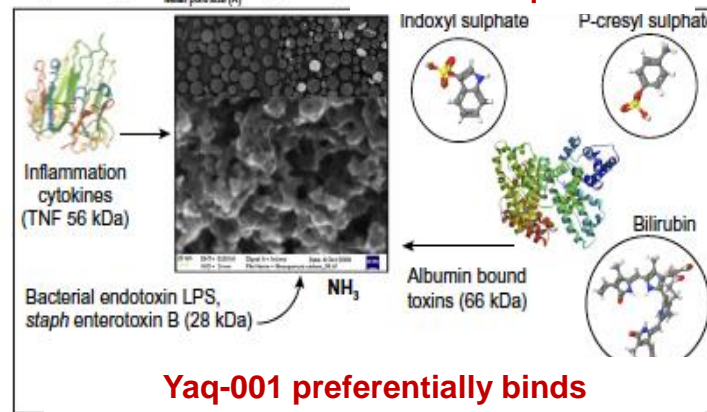
Manufacturing process makes bimodally porous carbons that have both micropores and macropores



Physical appearance of Yaq-001

... macroporosity delivers the power to absorb large molecules

Yaq-001 is insoluble. It is excreted with bound molecules attached.



Yaq-001 preferentially binds hydrophobic compounds such as LPS

In bile-duct ligated cirrhotic rats, Yaq-001 (4 g/kg, 2 wk, po):

- Improved liver function tests and reduced portal pressure
 - No changes in liver fibrosis
- Prevented LPS induced ACLF
- Normalized the Bacteroides/Firmicutes ratio, improved Paneth cell function and reduced gut bacterial translocation
 - No adverse effects on the gut mucosa

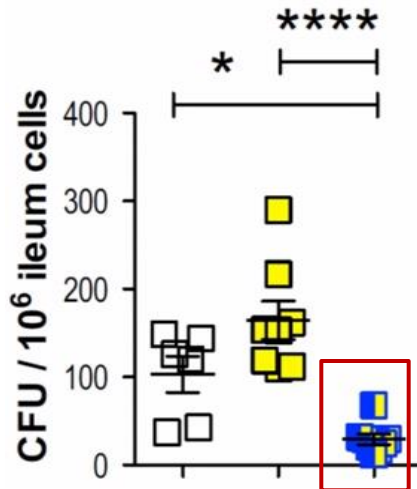
J Macnaughtan et al. Gut 2015

EC H2020

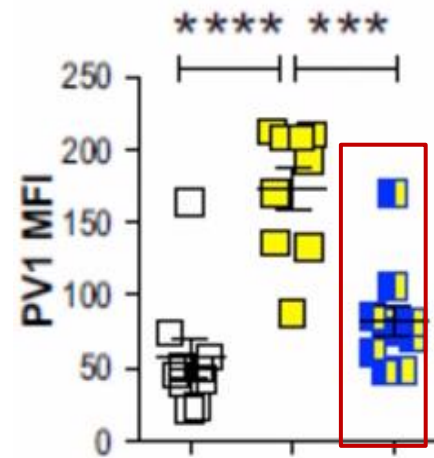
- Safety and Tolerability of Yaq-0001 in patients with cirrhosis (CARBALIVE SAFETY)
- Yaq-0001 to prevent cirrhosis-related complications (PREVENT-ACLF)
- Yaq-0001 in NASH (TREAT-NAFLD)

Obeticholic acid restores MCDD-induced dysruption of the gut-vascular barrier

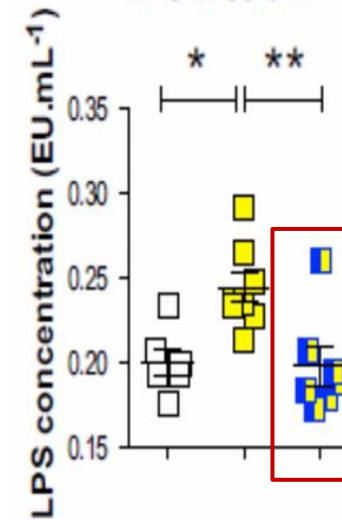
Bacteria attached to the mucosa



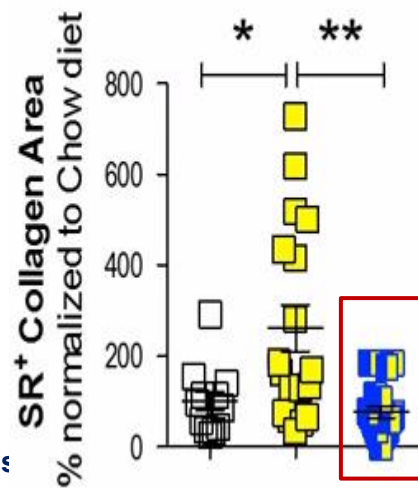
Ileum PV1 expression



LPS in serum



Fibrosis



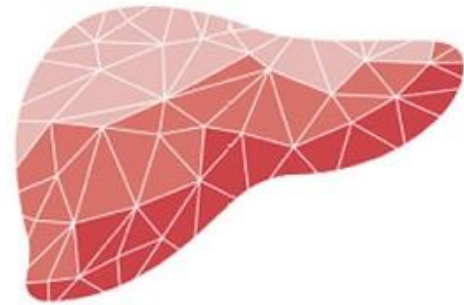
CHOW
 MCDD
 MCDD OCA

“Eje hígado-intes-

ica hepática por grasa”

L Adorini, Mouries et al. EASL Leuven MTC 2018

- MAFLD is associated (by a HFD?) with an altered composition and function of the intestinal microbiota
- Dysbiosis mediates damage in the epithelial and vascular gut barriers induced by a low-fiber/high-fat diet
- Metabolites produced by gut microbiome contribute to liver damage in MAFLD and might serve as targets of therapy and biomarkers of disease



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