Role of the microbiome in NASH

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Disclosures

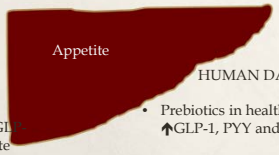
* Member of Nutricia's Speaker's Bureau

Learning objectives

* Analyzing the effects of the intestinal microbiota on nutrient metabolism
* Evaluating the inflammatory and pro-fibrotic impact of the microbiota on the liver of patients with non-alcoholic steatohepatitis (NASH)
* Demonstrating the therapeutic potential of the microbiota in NASH

Intestinal microbiota & steatosis

Appetite regulation



ANIMAL DATA

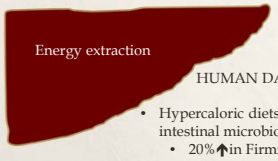
- Prebiotics ↑the release of anorexigenic peptides (e.g. GLP-1, PYY) and ↓ that of appetite enhancers (e.g. ghrelin)
- This is associated with decreased oral intake & weight loss

HUMAN DATA

- Prebiotics in healthy adults: ↑GLP-1, PYY and ↓appetite
- Endotoxin (LPS) levels are independently associated with caloric intake

Cani *et al. Am J Clin Nutr* 2009; Parnell *et al. Am J Clin Nutr* 2009; Reimer *et al J Proteome Res* 2012 ; Amar *et al. Am J Clin Nutr* 2009

Energy salvage from diet



ANIMAL DATA

- Carbohydrate fermentation ↑in *ob/ob mice*
- Fermentation ↑also with conventionalization of germ-free mice

HUMAN DATA

- Hypercaloric diets → shifts in intestinal microbiota:
  - 20%↑in Firmicutes leads to ↑energy harvest of 150 kcal
  - 20% ↑in Bacteroidetes leads to ↓energy harvest of 150 kcal
- Microbiome: ↑genes involved in CHO processing

Turnbaugh *et al. Nature* 2006; Turnbaugh et al. *Nature* 2009; Jumpertz *et al. Am J Clin Nutr* 2011

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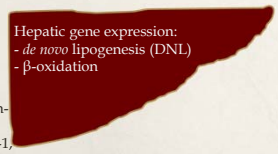
Hepatic Gene Expression

ANIMAL DATA

- Conventionalization of germ-free mice:
  - ↑ expression of SREBP-1, ChREBP
  - ↓AMPK activation
- Antibiotics to *ob/ob* mice ↓ steatosis, associated with ↓DNL &↑β-oxidation gene expression



Backhed *et al. Proc Natl Acad Sci U S A.* 2004; Membrez et al. *FASEB J* 2008

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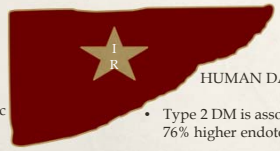
Insulin Resistance

ANIMAL DATA

- Endotoxin infusion → hepatic steatosis and IR
- Prebiotics → ↑Bifidobacteria → prevent high-fat diet induced IR (and endotoxemia)
- Antibiotics to high-fat diet fed mice → improved IR & attenuated inflammation in adipose tissue



HUMAN DATA

- Type 2 DM is associated with 76% higher endotoxin levels
- Endotoxin infusion to healthy adults → ↓IRS-1 expression and induction of IR

Cani et al, *Diabetes* 2007; Cani et al, *Diabetologia* 2007; Carbahlo *et al. Diabetologia* 2012; Creely *et al. Am J Physiol Endocrinol Metab* 2007 ; Mehta *et al. Diabetes* 2010

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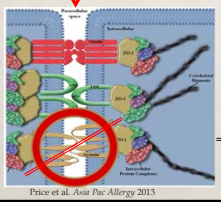
Intestinal Permeability

ANIMALS:
High Fat Diet →↓Lactobacilli

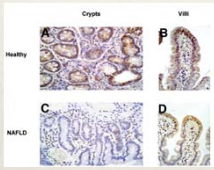
ANTIBIOTICS AND PREBIOTICS REVERSE HFD-INDUCED CHANGES IN INTESTINAL PERMEABILITY & MESENTERIC INFLAMMATION



Price et al. *Asia Pac Allergy* 2013

↑LPS and mesenteric cytokines

HUMANS:
NAFLD is associated with:
- ↓duodenal expression of tight junction proteins
- Small intestinal bacterial overgrowth



Cani *et al. Diabetes* 2008; Cani *et al. Gastroenterol* 2009; Lam *et al. PLoS One* 2012; Miele *et al. Hepatology* 2009; Abu Shanab *et al. Dig Dis Sci* 2011

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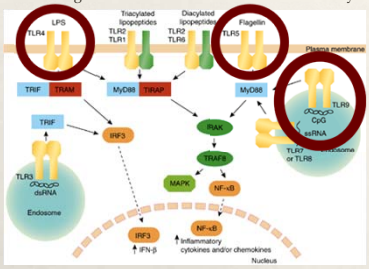
Toll-like Receptors

- Mice on NASH-inducing diets are protected if TLR4$^{-/-}$ or TLR9$^{-/-}$
- TLR5$^{-/-}$ → changes in intestinal microbiota → metabolic syndrome



Schmidt, C. Nature Biotechnol 2007

Rivera *et al. J Hepatol* 2007; Muira et al. *Gastroenterol* 2010; Vijay-Kumar *et al. Science* 2010; Karczewski *et al. Am J Physiol Gastrointest Liver Physiol.* 2010

L. plantarum to healthy adults ↑TLR2-dpd occludin deposition at tight junctions

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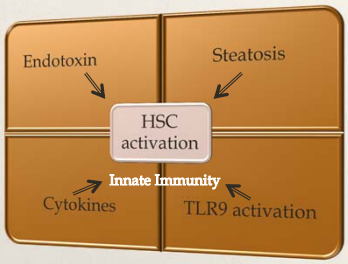
Microbiota & hepatic fibrosis



Seki *et al. J Physiol* 2012; Zhu *et al. J Hepatol* 2012; Seki *et al, Nat Med* 2007

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Intestinal microbiota & SCFA

Butyrate stimulates leptin and GLP-1 synthesis

Appetite
Kcal intake

SCFA contribute to adipocity & hepatic steatosis

Steatosis/Energy
SCFA R$^{-/-}$ →↓adiposity
Germ-free rats excrete x2 the amount of kcal in their stool

SCFA prevent activation of NF-κB

Inflammation
TNFα, IL-1β, IL-6

Butyrate: gene expression

Colonic integrity
Bacterial translocation

Bergman *et al. Physiol Rev* 1990; Wostmann *et al. Lab Anim Sci* 1983; Samuel *et al. Proc Natl Acad Sci USA* 2008;

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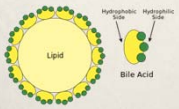
Intestinal Microbiota & Bile Acids

Effects of Bacteria on Bile Acids

- Primary Bile Acids: synthesized in the liver
  - Bacteria play a role via FXR activation
- Conjugated in liver with glycine (taurine in mice)
  - improved bioavailability
  - **bile salt hydrolases, widespread among intestinal bacteria, deconjugate bile acids**
- Secondary: **synthesized in the gut by bacteria-driven reactions** on primary bile acids that escape absorption

Effect of Bile Acids on Bacteria

- Bile acids act as detergents on bacterial cell membranes
  - Deoxycholic acid: most potent antibacterial properties
- Rats fed cholic acid → expansion of Firmicutes, esp. *Clostridia*



Sayin *et al. Cell Metab* 2013; Begley *et al. FEMS Microbiol Rev* 2005; Ridlon *et al. Gut Microbes* 2013; Ridlon et al. *Curr Opin Gastroenterol* 2014

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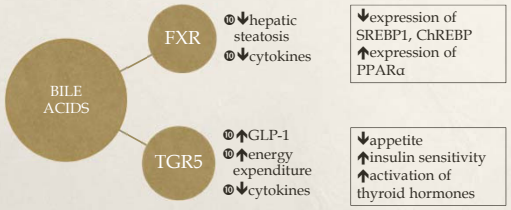
Bile Acids



Phase-2 trial: FXR agonists ameliorate insulin sensitivity and NASH

Pineda *et al. Mol Endocrinol* 2003; Wu *et al. Biochem Biophys Res Commun* 2014; Li *et al. Am J Physiol Gastrointest Liver Physiol* 2013; Thomas *et a. Cell Metabol* 2009; Watanabe *et al. Nature* 2006

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Evidence of therapeutic potential

Animal data:

Study	NASH model	Treatment	Effect
Raso *et al. J Nutr Biochem* 2014	High-fat diet (rats)	*L. paracacei* + arabinogalactan + FOS	↓steatosis, inflammation, insulin resistance, intestinal permeability
Wagnerberger *et al. J Nutr Biochem* 2013	Fructose (mice)	*L. casei Shirota*	↓steatosis, ALT and TLR4 activation in liver
Okubo *et al. Am J Physiol Gastrointest Liver Physiol 2013*	Methionine-choline deficient diet (mice)	*L. casei Shirota*	↓ hepatic and colonic inflammation/fibrosis, serum LPS
Ritze *et al. PLoS One* 2014	Fructose (mice)	*L. rhamnosus GG*	↓steatosis, ALT and improved duodenal tight-junction concentration
Pachikian *et al. Mol Nutr Food Res* 2013	N-3 PUFA-depleted diet (mice)	*FOS*	↓steatosis and ↑GLP-1
Endo *et al. PLoS One* 2013	Choline-deficient/L-amino acid defined diet (rats)	MIYAIRI 588 (butyrate producing)	↓steatosis, IR, improved tight-junction localization, ↑antioxidative enzymes

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Human data on treatment

- Meta-analysis of 4 RCTs (1 pediatric) including 134 patients with NAFLD/NASH show that probiotics:
  - ↓ ALT (4 studies)
  - ↓ AST, total cholesterol, TNF-α (3 studies)
  - ↓HOMA-IR (2 studies)
- Adult studies
  - VSL#3: n=22 x 12 weeks: ↓ ALT, AST, GGT and markers of lipid peroxidation
  - Synbiotic: n=52 x 28 weeks: ↓ ALT, AST, GGT and fibrosis (TE)

Ma et al. World J Gastroenterol 2013; Loguercio *et al. J Clin Gastroenterol* 2005; Eslambarast *et al. Am J Clin Nutr 2014*

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Future directions

- Describe the dysbiosis of patients with NAFL vs. NASH to guide the treatment strategies
  - Adults with NASH: ↓Bacteroidetes, *Faecalibacterium*
  - Children with NASH: ↑Proteobacteria (*E. coli*)
- Therapeutic potential of Bile Acids – FXR agonists
- Role of fecal transplantation

Mouzaki *et al. Hepatology* 2013; Zhu *et al. Hepatology* 2013; Wong *et al. PLoS One* 2013

Conclusions

- Intestinal microbiota participate in the development of steatosis, hepatic inflammation and fibrosis
- Modulation of bacterial composition may aid in the management of patients with NAFLD