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: CLP-1, PYY and appetite
- Endotoxin (LPS) levels are independently associated with caloric intake

Energy salvage from diet

ANIMAL DATA
- Carbohydrate fermentation: in ob/ob mice
- Fermentation also with conventionalization of germ-free mice

HUMAN DATA
- Hypercaloric diets shift 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
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

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

Intestinal Permeability

**ANIMALS:** High Fat Diet → Lactobacilli
**HUMANS:** NAFLD is associated with:
- Duodenal expression of tight junction proteins
- Small intestinal bacterial overgrowth

Toll-like Receptors

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

Microbiota & hepatic fibrosis

- Endotoxin → Steatosis
- HSC activation
- Cytokines
- TLR9 activation

Butyrate stimulates leptin and GLP-1 synthesis
- SCFA R-/- → adiposity & hepatic steatosis
- SCFA B-/- adiposity
- Germ-free rats excrete 2x the amount of kcal in their stool
- TNFα, IL-1β, IL-6
- Cytokine/gene expression
- Bacterial translocation

Microbiota & SCFA

- SCFA/Bile acids
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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

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**Microbiota & NASH treatment**

**Evidence of therapeutic potential**

<table>
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<tr>
<th>Study</th>
<th>NASH model</th>
<th>Treatment</th>
<th>Effect</th>
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<tr>
<td>Raso et al. J Nutr Biochem 2014</td>
<td>High-fat diet (rats)</td>
<td>L. paracasei + arabinogalactan + FOS</td>
<td>Steatosis, inflammation, insulin resistance, intestinal permeability</td>
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<tr>
<td>Wagenerberger et al. J Nutr Biochem 2013</td>
<td>Fructose (mice)</td>
<td>L. casei Shirota</td>
<td>Steatosis, ALT and TLR4 activation in liver</td>
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<td>Ohkawa et al. J Physiol Gastrointest Liver Physiol 2013</td>
<td>Methionine-choline-deficient diet (mice)</td>
<td>L. casei Shirota</td>
<td>Hepatic and colonic inflammation/fibrosis, serum IL-1β</td>
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<tr>
<td>Rasu et al. Proc Natl Acad Sci USA 2014</td>
<td>Fructose (mice)</td>
<td>L. rhamnosus GG</td>
<td>Steatosis, ALT and improved duodenal tight-junction concentration</td>
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<tr>
<td>Pichler et al. Mol Nutr Food Res 2013</td>
<td>N3 PUFAs-depleted diet (mice)</td>
<td>FOS</td>
<td>Steatosis and GLP-1</td>
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<td>Fedorova et al. J Lipid Res 2013</td>
<td>Choline-deficient/L-amino acid defined diet</td>
<td>MII-Agari 588 (butyrate producing)</td>
<td>Steatosis, IR, improved tight-junction localization, antioxidative enzymes</td>
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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
  - Visfatin n=22 x 12 weeks: ALT, AST, GGT and markers of lipid peroxidation
  - Symbiotic n=52 x 28 weeks: ALT, AST, GGT and fibrosis (TE)

**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

**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