Dysbiosis in obesity

Obesity:
- Dysbiosis, shown in multiple settings
- Fecal transplantation → transferable phenotype

Tilg et al. Curr Opin Pediatr 2015
Walker et al. Science 2013

Dysbiosis in NASH

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Methodology</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhu et al. Hepatol 2013</td>
<td>63</td>
<td>16S rRNA gene sequencing</td>
<td>• Proteobacteria in NASH&gt;obesity&gt;lean</td>
</tr>
<tr>
<td>Michail et al. FEMS Microbiol 2015</td>
<td>50</td>
<td>16S rRNA gene sequencing</td>
<td>• Gammaproteobacteria, • Prevotella (Bacteroidetes)</td>
</tr>
<tr>
<td>Mouzaki et al. Hepatol 2013</td>
<td>50</td>
<td>PCR</td>
<td>• Bacteroidetes in NASH vs. NAFL/controls</td>
</tr>
<tr>
<td>Raman et al. Citi Gastroenterol Hepatol 2013</td>
<td>60 adults</td>
<td>Multitag pyrosequencing</td>
<td>• Lachnospiraceae (Firmicutes), • Gammaproteobacteria</td>
</tr>
<tr>
<td>Spencer et al. Gastroenterol 2011</td>
<td>15 healthy women; choline depletion diet</td>
<td>16S rRNA gene sequencing</td>
<td>• Baseline Gammaproteobacteria protective</td>
</tr>
</tbody>
</table>

Pathogenesis

Dietary intake

- Appetite
- Calorie extraction
- Gene expression
- Inflammation

Ethanol
SCFA
Bile acids

Intestinal microbiota

Intestinal permeability

NASH
Increased intestinal permeability in pediatric NAFLD:
- Lactulose/mannitol permeability
- Circulating zonulin levels
- Increased circulating LPS levels


Adapted from: Paolella et al. *World J Gastroenterol* 2014

Impaired gut barrier in NAFLD

Impaired tight junctions

Atrophy of microvilli (and disruptive tight junctions)


Gut barrier: microbiota and intestinal mucus layer

<table>
<thead>
<tr>
<th></th>
<th>Room 1</th>
<th>Room 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td>Heavier</td>
<td>Leaner</td>
</tr>
<tr>
<td>16S rRNA</td>
<td>Anaerostipes genus (Clostridia class)</td>
<td>Proteobacteria and TM7 phyla</td>
</tr>
<tr>
<td>Mucus properties</td>
<td>Impenetrable</td>
<td>Penetrable</td>
</tr>
</tbody>
</table>

- Fecal transplantation: similar changes in mucus
- *Akkermansia muciniphila* restores mucus thickness in obesity

Indirect effects of intestinal microbiota on the gut barrier

- Bile acids
  - In animal model of fructose-induced NASH:
    - Normalization of occludin levels in duodenum
    - Decreased endotoxemia
    - Decreased TNFα expression in liver

- Ethanol

Ethanol and gut barrier

- Tight junctions:
  - In healthy humans 20g x1 → decreased occludin and zo-1 expression in duodenum

- Mucus layer:
  - Ethanol dissolves lipids from the mucus layer → loss of hydrophobicity

- Aldehyde dehydrogenase polymorphisms (?): increased acetaldehyde → disruption of tight/adherence junctions → liver injury

Volynets et al., J Lip Res 2010; Elamin et al., PLoS One 2014; Win et al., Toxicol Lett 2015; Chaudhry, Alcohol Clin Ex Res 2015
Endotoxemia

- Described in adults and children with NAFLD
- LPS, LBP, LPS IgG, sCD14

Endotoxemia has been associated with:
- Fructose intake, high fat diet
- Insulin resistance
- Hepatic inflammation


LPS and metabolic dysregulation

LPS and metabolic dysregulation

Dysbiosis, endotoxemia and hepatic fibrosis

Control diet | High fat diet | Control diet + BDL | High fat diet + BDL
---|---|---|---
- HFD+BDL: decreased bacterial diversity
- HFD flora to control diet mice + BDL → hepatic fibrosis
- Gram negative to control diet mice+ BDL → hepatic fibrosis

De Minicis et al. Hepatol 2014

Caricilli et al. Nutrients 2013
Intestinal fatty acid composition determines endotoxemia

Kallianan et al. Sci Rep 2015

Transgenic mice able to convert n-6 PUFA to n-3
All mice on same diet
Western diet n6:n3 = 10:1
Healthy diet n6:n3 ~ 1:1

Dysbiosis & leaky gut lead to NAFLD

NASH

Microbial byproducts and steatohepatitis: ethanol

- ethanol: NASH=ASH?
- Blood ethanol levels are higher in adults and children with NAFLD
- Increased numbers of bacteria that synthesize ethanol (e.g. E. coli) in children with NASH
### Microbial byproducts and steatohepatitis

<table>
<thead>
<tr>
<th>Ethanol</th>
<th>SCFA</th>
<th>Bile acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient handling</td>
<td>Intestinal permeability</td>
<td>Increased calorie extraction</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>Inhibition of insulin signaling</td>
<td>↑GLP-1 synthesis</td>
</tr>
<tr>
<td>Steatosis</td>
<td>↑fat oxidation</td>
<td>Effects on appetite, de novo lipogenesis</td>
</tr>
<tr>
<td>Hepatotoxicity</td>
<td>Direct hepatotoxicity</td>
<td>-</td>
</tr>
<tr>
<td>Immunity</td>
<td>Dysfunction of CD4+ T cells</td>
<td>Multiple effects</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>Activation of stellate cells</td>
<td>-</td>
</tr>
</tbody>
</table>


Pediatric NAFLD: beyond dysbiosis

- Phylogenetic data:
  - Gammaproteobacteria & Prevotella
- Metabolomic data:
  - Ethanol and acetate
- Metagenomic and metaproteomic data:
  - Energy metabolism
  - Lipid synthesis
  - Ethanol synthesis
  - Fewer pathways for carbohydrate and a.a. metabolism

Diet and exercise: are we just changing the bacteria?

Exercise improves NAFLD

Exercise is associated with increased bacterial diversity

Conclusions

- Intestinal microbiota are involved in the pathogenesis of NAFLD
- Impaired gut barrier and endotoxemia play a crucial role
- Advanced technology should be used to further our understanding
- Modulation of products of bacterial metabolism may confer beneficial results
Thank you

Other microbial products involved in immune responses

- **Bile acids**: FXR, TGR5 on innate immune cells → anti-inflammatory effects
- **SCFA**: 
- **Ethanol**: dysfunction of CD4+ T cells

VanBest et al. Hepatol Int 2015
Engstler et al. Gastroenterol 2015
ADH activity is decreased in ob/ob mice
TNF plays a role in ADH activation
Ethanol levels are increased in ob/ob mice
Intestinal microbiota and immunity: a vicious cycle

- Dysbiosis
- Immune activation
- Impaired gut barrier

Immune dependent regulation of intestinal microbiota composition

- TLR5<sup>−/−</sup>: hyperphagia, metabolic syndrome, dysbiosis; phenotype is transferable
- Inflammasome deficiency: progression to NASH associated with dysbiosis - colitogenic IM composition; phenotype is transferable
- CX3CR1 deficiency: Increased intestinal permeability, steatohepatitis, insulin resistance and dysbiosis

• Weight loss
• Increased Fiaf
• Inefficient emulcification of fat due to unconj BA?
• Decreased cholesterol
• Increased REGIIIγ
• Antibacterial

• Increased 2ndary bile acids → hepatotoxicity, HCC

Joyce et al., Gut Microbes 2014; Joyce et al., Proc Natl Acad Sci USA 2014; Yoshimoto et al., Nature 2013

Schneider et al., Hepatology 2015

Vijay-Kumar et al., Science 2010; Henao-Mejia et al., Nature 2012