STOPNASH: Microbiome and NAFLD in children

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Disclosures

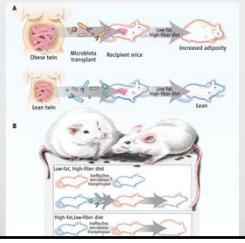
- Nothing to disclose

Outline

- Dysbiosis and the gut barrier
- Endotoxemia and the liver
- Impact of products of bacterial metabolism

Dysbiosis in obesity

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



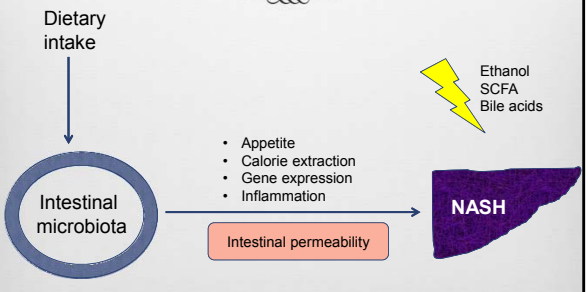
Tilg et al. *Curr Opin Pediatr* 2015; Walker et al. *Science* 2013

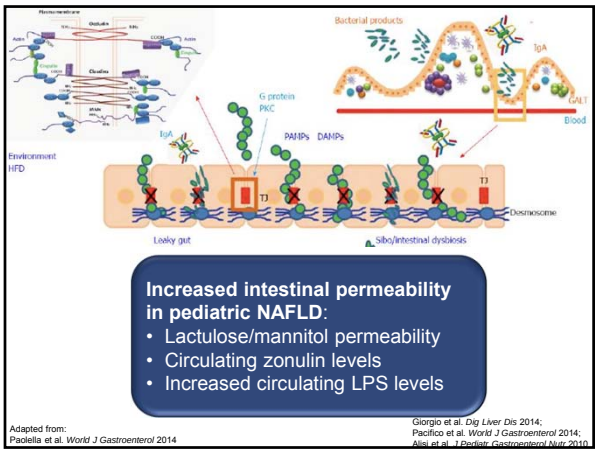
Dysbiosis in NASH

Study	N	Methodology	Result
Zhu et al. *Hepatol* 2013	63 children	16S rRNA gene sequencing	↑Proteobacteria in NASH>obesity>lean
Michail et al. *FEMS Microbiol Ecol* 2015	50 children	16S rRNA gene sequencing	↑Gammaproteobacteria ↑Prevotella (Bacteroidetes)
Mouzaki et al. *Hepatol* 2013	50 adults	PCR	↓Bacteroidetes in NASH vs. NAFL/controls
Raman et al. *Clin Gastroenterol Hepatol* 2013	60 adults	Multitag pyrosequencing	↑Lachnospiraceae (Firmicutes) ↑Gammaproteobacteria
Spencer et al. *Gastroenterol* 2011	15 healthy women; choline depletion diet	16S rRNA gene sequencing	↑baseline Gamma-proteobacteria protective

Ferolla et al. *World J Hepatol* 2015

Pathogenesis





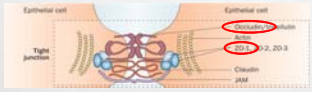
Increased intestinal permeability in pediatric NAFLD:

- Lactulose/mannitol permeability
- Circulating zonulin levels
- Increased circulating LPS levels

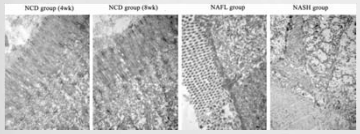
Adapted from:
Paolella et al. *World J Gastroenterol* 2014

Giorgio et al. *Dig Liver Dis* 2014;
Pacifico et al. *World J Gastroenterol* 2014;
Alisi et al. *J Pediatr Gastroenterol Nutr* 2010

Impaired gut barrier in NAFLD



Impaired tight junctions



Atrophy of microvilli
(and disruptive tight junctions)

Jiang et al. *Sci Rep* 2015;
Miele et al. *Hepatology* 2009;
Neunlist et al. *Nat. Rev. Gastroenterol. Hepatol* 2012;
Mao *Int J Clin Exp Pathol* 2015

Gut barrier: microbiota and intestinal mucus layer

	Room 1	Room 2
Mice	Heavier	Leaner
16S rRNA	↑Anaerostipes genus (Clostridia class) ↑Erysipelotrichi class	↑Proteobacteria and TM7 phyla
Mucus properties	Impenetrable	Penetrable

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

Jakobsson et al. *EMBO Rep* 2015;
Everard et al. PNAS 2013

Indirect effects of intestinal microbiota on the gut barrier

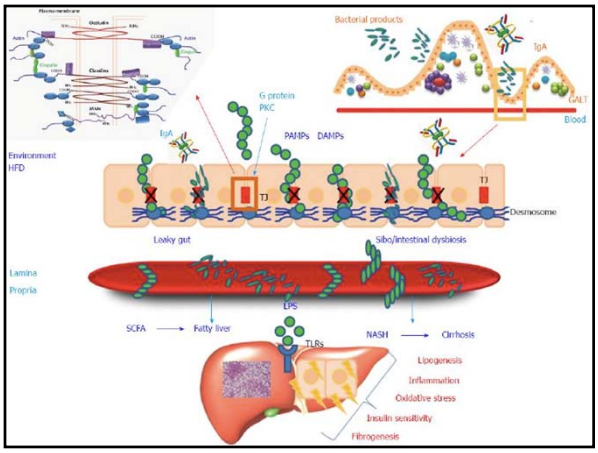
- Bile acids
  - In animal model of fructose-induced NASH:
    - Normalization of occludin levels in duodenum
    - Decreased endotoxemia
    - Decreased TNFα expression in liver
- Ethanol

Volynets et al. *J Lip Res* 2010; Elamin et al. *PLoS One* 2014; Win et al. *Toxicol Lett* 2015

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

Elamin et al. *PLoS One* 2014; Win et al. *Toxicol Lett* 2015; Chaudhry, *Alcohol Clin Ex Res* 2015

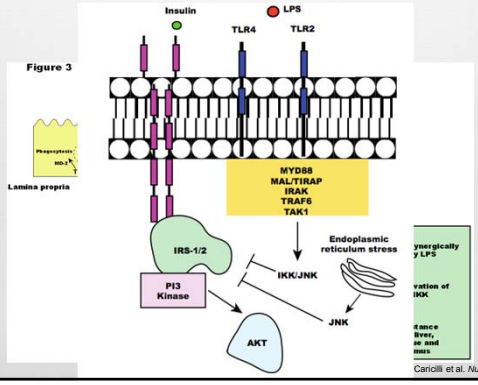


Endotoxemia

- 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

Harte et al. *J Inflamm* 2010;
Ogawa et al. *PLoS One* 2013;
Verdam et al. *J Clin Gastronterol* 2011;
Thuy et al. *J Nutr* 2008;
Pendyala et al. *Gastroenterol* 2012
Ruiz et al. *Obes Surg* 2007;
Jin *Int J Hepatol* 2014

LPS and metabolic dysregulation

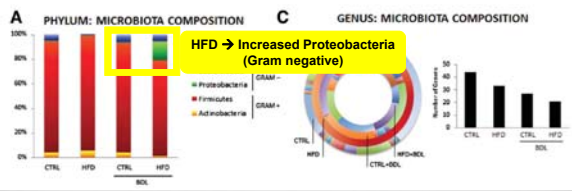


Caricilli et al. *Nutrients* 2013

Dysbiosis, endotoxemia and hepatic fibrosis

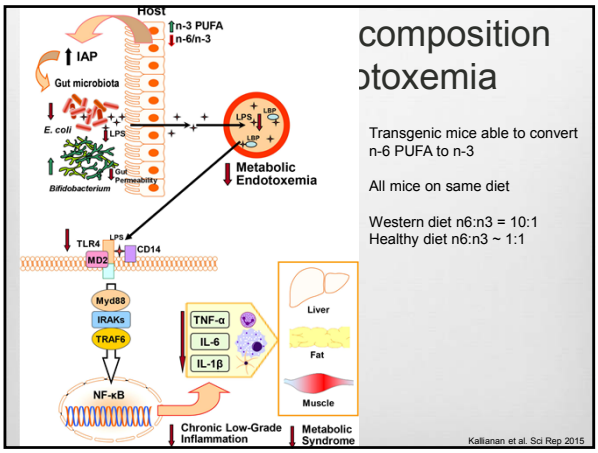
Control diet	High fat diet	Control diet + BDL	**High fat diet +BDL**

- 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



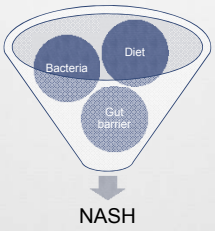
composition otoxemia

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

Inflammation → #ADH activity → ↑Ethanol

Volynets et al. *Dig Dis Sci* 2012;
Zhu et al. *Hepatology* 2012;
Michail et al. *FEMS Microbiol Ecol* 2015;
Nair et al. *Am J Gastroenterol* 2001;
Engstler et al. *Gastroenterol* 2015

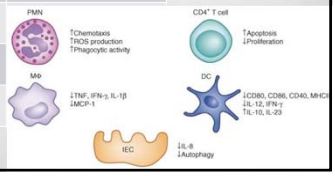
Microbial byproducts and steatohepatitis

	Ethanol*
Nutrient handling	Intestinal permeability
Insulin resistance	Inhibition of insulin signaling
Steatosis	↓fat oxidation
Hepatotoxicity	Direct hepatotoxicity
Immunity	dysfunction of CD4+ T cells
Fibrosis	Activation of stellate cells

Chen et al. *Int J Mol Med* 2015;
Shelmet et al. JCI 1988;
Ghare et al. *Alcohol Clin Exp Res* 2011
Reyes-Gordillo et al. *Am J Pathol* 2014;

Microbial byproducts and steatohepatitis

	Ethanol*	SCFA
Nutrient handling	Intestinal permeability	Increased calorie extraction
Insulin resistance	Inhibition of insulin signaling	↑GLP-1 synthesis
Steatosis	↓fat oxidation	Effects on appetite, *de novo* lipogenesis
Hepatotoxicity	Direct hepatotoxicity	
Immunity	dysfunction of CD4+ T cells	
Fibrosis	Activation of stellate cells	



Hosseini et al. *Nutr Rev* 2011;
Vinolo et al. *Nutrients* 2011

Microbial byproducts and steatohepatitis

	Ethanol*	SCFA	Bile acids
Nutrient handling	Intestinal permeability	Increased calorie extraction	Fat digestion, metabolic rate
Insulin resistance	Inhibition of insulin signaling	↑GLP-1 synthesis	↑GLP-1 release
Steatosis	↓fat oxidation	Effects on appetite, *de novo* lipogenesis	Regulation of fat handling
Hepatotoxicity	Direct hepatotoxicity	-	LCA hepatotoxicity, DCA ROS/HCC
Immunity	dysfunction of CD4+ T cells	Multiple effects	Antimicrobial: innate immunity**
Fibrosis	Activation of stellate cells	-	Activation of stellate cells

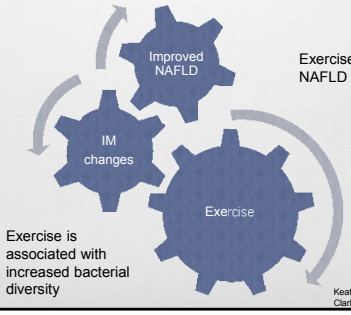
Watanabe et al. *Nature* 2006;
Woolbringht et al. *Toxicol Lett* 2014;
Yoshimoto et al. *Nature* 2013;
Ramm et al. *Hepatology* 2009

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

Michail et al. *FEMS Microbiol Ecol* 2015

Diet and exercise: are we just changing the bacteria?



Exercise improves NAFLD

Exercise is associated with increased bacterial diversity

Keating et al *J Hepatol* 2015; Clarke et al. *Gut* 2014

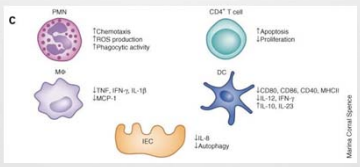
Conclusions

- 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

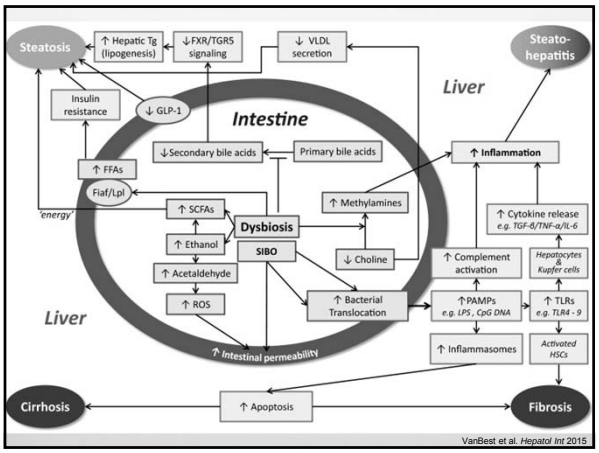
Other microbial products involved in immune responses

- **Bile acids**: FXR, TGR5 on innate immune cells→ anti-inflammatory effects
- **SCFA**:

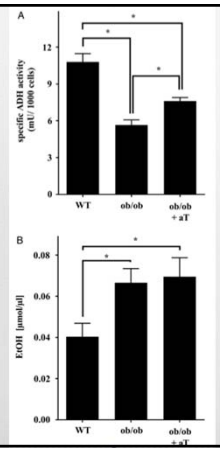


- **Ethanol**: dysfunction of CD4+ T cells

Ghare et al. *Alcohol Clin Exp Res* 2011;
Brestoff et al. *Nat Immunol* 2013;



VanBest et al. *Hepatol Int* 2015

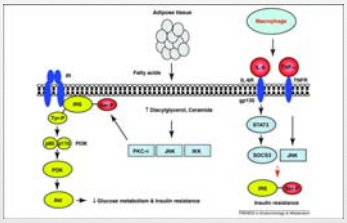


ADH activity is decreased in ob/ob mice

TNF plays a role in ADH activation

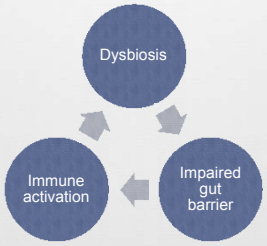
Ethanol levels are increased in ob/ob mice

Engstler et al. Gastroenterol 2015



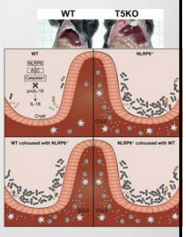
Gray et al. Trends in Endocrinology & Metabolism 2001

Intestinal microbiota and immunity: a vicious cycle

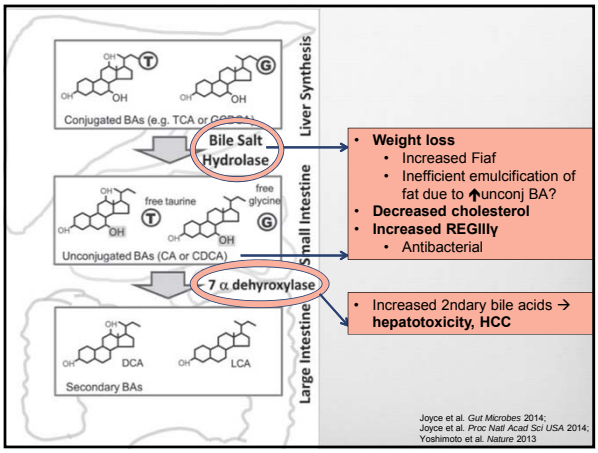


Immune dependent regulation of intestinal microbiota composition

- **TLR5**$^{-/-}$: 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



Vijay-Kumar et al. *Science* 2010; Henao-Mejia et al. *Nature* 2012; Schneider et al. *Hepatology* 2015



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